DESIGN AND DEVELOPMENT OF LABORATORY SCALE HYDROPONIC SYSTEM FOR GROWING

SWEET BASIL USING PLASMA ACTIVATED NUTRIENT SOLUTION

By

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A thesis submitted to the

School of Graduate Studies

Rutgers, The State University of New Jersey

In partial fulfillment of the requirements

For the degree of

Master of Science

Graduate Program in Food Science

Written under the direction of

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New Brunswick, New Jersey

May 2020

ABSTRACT OF THE THESIS

Design and development of laboratory scale hydroponic system for growing sweet basil using plasma activated nutrient solution

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Food security for future generations has become a critical issue for a variety of reasons such as population explosion, climate change, and less land for crop cultivation. Alternative growing techniques such as hydroponics which do not involve the use of soil for crop production can be used on commercial level to alleviate the food security problem. In our research, sweet basil was grown using plasma activated nutrient solution (PANS) in a closed hydroponic system. The PANS offers number of benefits over nutrient solution (NS) for basil growth due to the presence of reactive oxygen and nitrogen species. PANS was prepared using a gliding arc plasmatron system developed by Drexel University and was characterized by measuring its pH, EC (electrical conductivity), ORP (oxidation-reduction potential), and reactive species concentrations.

To assess the effect of PANS on sweet basil plant growth and quality, basil seedlings were grown using Rutgers Devotion DMR cultivar seeds in a growth chamber for 14 days (25 °C, 75 % relative humidity, and 100 µmol/m^{2}s light intensity). These seedlings were then transferred

to custom-built NS and PANS chambers for 20 days growth period. The two chambers were identical and were assembled in the pilot plant of Rutgers Food Science building. The plants received fixed amount of light (250 µmol/m^{2}s) for 16 h each day and they were watered 8 times a day for 8 mins at a time, throughout the harvest period. The temperature, the relative humidity, the CO_2 level inside the two chambers, and the temperatures of NS and PANS were monitored every 1 h. To evaluate the effect of PANS, two treatment variations were done while growing the basil plants in the two chambers. In Treatment 1, the NS and PANS solutions that were prepared on day 1 of the harvest period were used throughout the 21-day growth period to water the basil plants whereas in Treatment 2, fresh NS and PANS solutions were used to water the plants for week 1, week 2, and week 3. For both the treatments, the basil plants were harvested on the 21st day and their growth parameters such as plant height, number of branches, number of nodes, root length, leaf index, mass yield, and quality parameters such as leaf color, texture, microbial quality, aroma profile, and tissue nutrient content were evaluated. The growth of algae in the solutions was also estimated at the end of every week.

Results showed that basil grown in PANS had higher growth and some improved quality parameters compared to basil grown using NS. PANS Treatment 1 (PANST1) had the most significant effect on the basil plants with increased height (11 %), more number of branches and nodes, and higher mass yield (35 %). PANS Treatment 2 (PANST2) resulted in basil leaves which had higher green (a*) value and higher leaf index. Peak rupture force, leaf toughness, and Young's modulus values for basil leaves were not significantly different between PANST1, PANST2, and control. Aroma analysis of the basil leaves showed that PANST1 resulted in an increased formation of methyl eugenol and eugenol, possibly indicating a spicier aroma from the leaves. Significant algae reduction (41 % and 45 %, respectively) was observed in PANST1 and PANST2 after first 2 weeks. However, at the end of week 3 only PANST2 showed significant algae

reduction (45 %). In terms of microbial quality, it was found that both PANST1 and PANST2

treatments did not show any significant difference in total plate count of the basil leaves, when

compared to control. The results from the tissue analysis indicated no difference in the tissue

nutrient profile for both the treatments when compared to control. Energy wise, the growth of

basil by control treatment required 73.9 kWh total energy, whereas PANST1 treatment required

74.9 kWh and PANST2 treatment required 76.9 kWh.

This study showed that growing Rutgers Devotion DMR sweet basil using PANS resulted

in higher (11 %) growth, higher yield (35 %), darker green color, and an altered aroma profile in

the basil leaves. Growing sweet basil under PANST1 was better for plant height, number of

branches, number of nodes, basil yield, and aroma profile. Use of PANST2 was better for

greener leaf color, higher leaf index, and more algae removal. Thus, use of PANS appears to

show a promise for growing basil hydroponically. Further research is needed for scaled-up

operations at a greenhouse scale.

ACKNOWLEDGEMENTS

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1. INTRODUCTION

Due to climate change, increasing population, and ever-increasing food demand, it has become necessary to use alternative growing practices such as hydroponics to grow fresh produce including herbs. Sweet basil (*Ocimum basilicum* L., Family Lamiacea) is one of the most widely grown herbs using hydroponics in the US (Walter and Currey, 2015). In this project, our aim was to explore and evaluate the effect of plasma to treat nutrient solution used in closed hydroponic system to water the plants on growth and quality of basil plants.

1.1 Soilless agriculture

Food security is a very critical issue which involves socioeconomic, agronomic, political, and environmental aspects. It is based on food availability, food access, and food use (Matuschke, 2009). Soil is one of the most valuable resource to ensure food security for future generations as it is the most common growing medium for plants (Tajudeen and Taiwo, 2018). It provides support, nutrients, air, and water which are essential for plant growth (Dholwani et al., 2018). Moving into the future this valuable resource has a lot of limitations in its use. Traditional agricultural practices are facing challenges like soil erosion, drought, desertification, flooding, and reduced soil fertility (Tajudeen and Taiwo, 2018). The world population is projected to reach 9.7 billion in 2050. Currently, 55% of the population resides in urban areas. By 2050, this number is projected to increase to 68% (United Nations, 2018). This population growth is resulting in increased uncontrolled urbanization, diminishing fresh water supplies, and decline in arable land per person. Threats like climate change, deteriorating fisheries, soil depletion is further going to affect available arable land. According to FAO, the arable land will decease to one third by 2050

of the available arable land in 1970 (Benke and Tomkins, 2017). These factors make sustainable

food production more complex and uncertain thus creating an urgent need to develop and

practice alternate sustainable agricultural practices such as hydroponics that can generate high

yields with reduced use of water and land (Singh et al., 2017).

1.1.1 Types of soilless agriculture

Soilless agriculture is interchangeably used with the word hydroponics. Three main

types of soilless agriculture are hydroponics, aeroponics, and aquaponics. Hydroponics involves

use of water and substrates to grow plants (explained in detail in section 1.1.2).

Aeroponics

In aeroponics (Figure *1*), plant roots are bathed using a nutrient water mist. The mist is

generated using misters or sprayers. This results in more aeration for roots as they are

suspended in the air. This method is widely used for growing lettuce and spinach (Khan et al.,

2018). The advantages of aeroponics technique are, space utilization is very efficient, double the

number of plants/unit area as compared to other hydroponic techniques can be cultivated

(Kaan, 2008), and least amount of water is used as compared to other hydroponic methods

(Savvas et al., 2013).
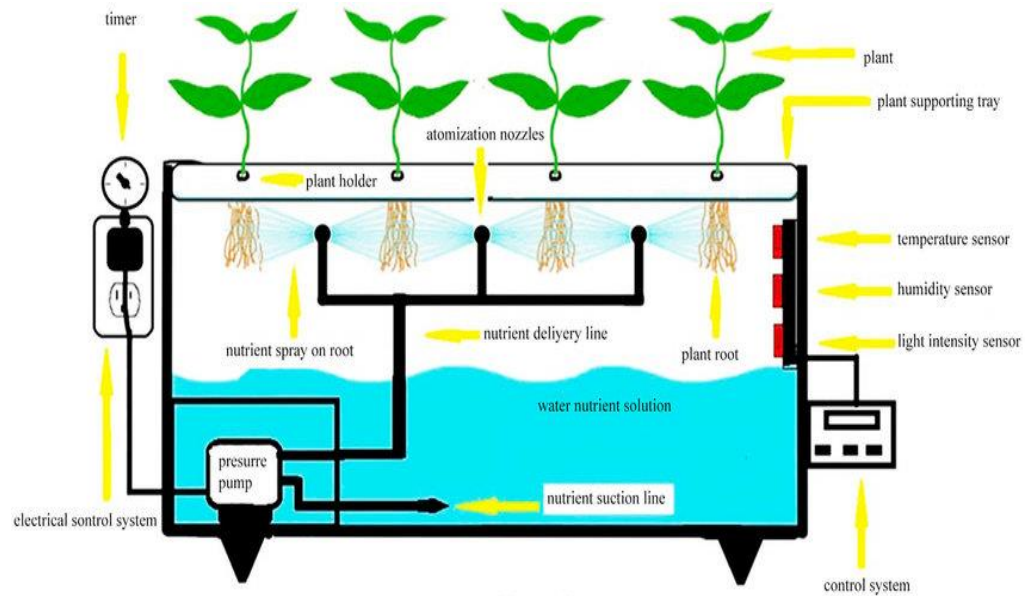
Figure 1: Aeroponics system (Source: Lakhiar et al., 2018).

Aquaponics

Aquaponics (Figure 2) is a combination of aquaculture and hydroponics. Aquaculture accounts for half of the fish production in the world (Somerville et al., 2014). In aquaponics, water from aquaculture where fish are grown is filtered using mechanical and biofilters where nitrifying bacteria are present and then used to grow crops. Excess water from the plants is filtered and used for hydroponics. This creates symbiotic relationship between plants, fish, and bacteria (El-Kazzaz and El-Kazzaz, 2017; Somerville et al., 2014).
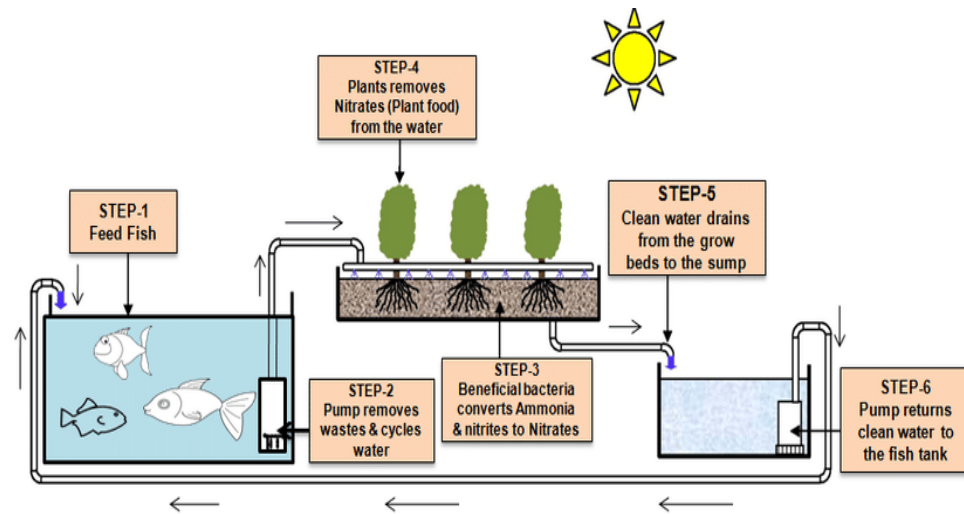
Figure 2: Depiction of aquaponics showing symbiotic relationship between plants, fish, and bacteria (Source: Jena et al., 2017).

Table 1: Comparison between soilless agriculture practices

Characteristic	Hydroponics	Aeroponics	Aquaponics
Root system	Periodically bathed in nutrient solution	Suspended mid air	Periodically bathed in aquaculture water
Water recirculation	Yes/no	Yes	Yes
Use of nutrient solution	Yes	Yes	No

Advantages	Fungicides and pesticides can be used	Fungicides and pesticides can be used	Production of fish and produce
Root disease occurrence	More	Less	Less
Technical know how	Low to moderate	High	High
Waste generation	Moderate to high	Moderate	Low
Type of crops grown	Wide range	Limited range	Limited range
Initial investment	Moderate to high	High	High
Commercial use	High	Increasing	Increasing
Reference	Roberto (2003)	Roberto (2003)	El-Kazzaz and El-Kazzaz (2017); Somerville et al. (2014)

1.1.2 Hydroponics: definition, history, and current use

The word hydroponics has its origin in the Greek language; ''hydro'' meaning water and ''ponos'' meaning labor. Hydroponics is defined as "the science of growing or the production of plants in nutrient-rich solutions and moist inert material such as gravel, vermiculite, saw dust instead of soil" (Jones, 2014). It makes use of liquid nutrient cultures rather than soil. It is one of

the many forms of soilless agriculture. Hydroponics has a very long history. The hanging gardens of Babylon is one of the great historical examples of hydroponic technology (Kaan, 2008; Rorabaugh et al., 2002). The first book on soilless agriculture *Sylva Sylvarum*wa was published by Francis Bacon in 1647. In 1880s German botanists made some progress in developing soilless culture technique making use of the water culture containing salts of macronutrients such as elemental N, P, Ca, Mg, and S (Khan et al., 2018). From 1880-1940, water culture was extensively studied and micronutrients necessary for plant growth were identified (Rorabaugh et al., 2002). In 1925, greenhouse industry expressed some interest in commercialization of the water culture. In early 1930s successful experiments on tomato vines were carried out at University of California by professor W. F. Gericke. He was able to grow 7.5 m height tomato vines (Giurgiu et al., 2014; Singh et al., 2017). The word hydroponics was first used by him in 1937 (Khan et al., 2018). This was the beginning of commercialization of hydroponic technology. After that, US army did hydroponic production of vegetables on pacific non-arable land during second world war to supply fresh vegetables to troops and civilians (Savvas, 2003). In 1950s hydroponics technology was mostly used in the European countries. It had limited acceptance in rest of world as it made use of expensive concrete beds for plant growth. Today, Holland, Japan, Canada, and the United States are top producers for hydroponic crops.

In hydroponics, the plant roots are hanging in a nutrient solution or bathed periodically with nutrient solution (Nguyen et al., 2016). Typically, hydroponic technique is practiced in greenhouses where controlled environment in terms of temperature, light, relative humidity, and carbon dioxide (CO_2) can be maintained and monitored making hydroponics a part of controlled environment agriculture. As compared to traditional open field agriculture, produce grown in controlled environment is regarded as safe due to its less exposure to environmental conditions (Gomez et al., 2019). Exposure of produce to soil borne pests and pathogens is

largely reduced (Kaan, 2008). Also, it uses 1/5th to 1/10th of the water used by traditional

cultivation practices (Kumari et al., 2018). Initially only herbs, tomatoes, and lettuce were grown

commercially using hydroponics (Jones, 2014). These days, wide variety of produce such as

pepper, spinach, strawberry, cucumber, green beans, and salad greens are grown

hydroponically (Jones, 2014; Stanghellini and Rasmussen, 1994). Lettuce and tomato are two

most widely cultivated hydroponic crops in the world (Aires, 2018). Barbosa et al. (2015)

compared the yield, energy requirement, and water use for lettuce production using

hydroponics and traditional growing methods in Arizona, USA. They found that hydroponic

method gave more than 10-fold yield, in less than 1/10th of the water as compared to traditional

method. But the energy requirement/kg of lettuce was more than 80 times that of traditional

method.

Table 2: Yield comparison between hydroponic system and open field system (Khan et al., 2018;
Jones, 2014).

Crop	Open field (kg/ha)	Hydroponic system (kg/ha)
Rice	841.03-1009.25	13456.56
Wheat	672.83	5606.90
Tomato	11203-22407.76	403335.81
Lettuce	10092.42	23548.98
Peas	2242.76	15699.32

Table 3: Water use in hydroponics and open field cultivation (Sambo et al., 2019)

Crop	Open field (L/kg)	Hydroponics (L/kg)
Lettuce	76	1.6
Hot pepper	110	58
Tomato	78	35
Strawberry	544	136

Water dependency of plant cultivation can be reduced significantly using hydroponic techniques (See Table 2 and

Table *3*). Also, any land that is not suitable for agricultural use due to inherent low quality, climate or overuse can be made use of for hydroponic crop cultivation. Thus, hydroponic cultivation of crops is very efficient and environmentally friendly alternative to traditional practices.

1.1.2.1 Advantages and limitations of using hydroponic systems

Advantages

- Laborious traditional practices such as watering, tilling, ploughing, weeding, fumigating are eliminated

- Improved productivity

- High density planting

- Soil borne plant diseases can be eliminated, good alternative to soil disinfection

- Urban agriculture is possible

- Protection of crops from extreme weather conditions

- Faster growth of herbs with more flavor and aroma

- Seasonal produce production can be done all year round

- No use of pesticides and herbicides

- Land and water conservation

(Tajudeen and Taiwo, 2018; Rorabaugh et al., 2002; Putra and Yulinado, 2003; Khan et al., 2018; Giurgiu et al., 2014; Dholwani et al., 2018)

Hydroponics certainly has more advantages than limitations. The main limitation being its high installation cost (Putra and Yulinado, 2003).

Limitations

- High level of maintenance and management

- Substrate disposal

- Not suitable for all types of crops

- Due to high cost, limited to high value crops

- Constant need of power to operate water and air pumps

(Pardossi et al., 2011; Hassall, 2001; Kaan, 2008; Tajudeen and Taiwo, 2018)

1.1.2.2 Types of hydroponic systems

Hydroponic systems can be broadly divided into free drain or open that is closed or recirculating systems. In closed system, the nutrient feed water is recovered (leachate) (Savvas et al., 2013), and recirculated to the plants (Figure 3) whereas in an open system this feed water is not reused, it is discarded (Figure 4) (Stanghellini and Rasmussen, 1994). Open systems are more used on commercial level as they are easy to manage (Pardossi et al., 2011). The main disadvantage of open systems is more water use whereas closed systems need frequent nutrient solution monitoring and adjustments (Carmassi et al., 2005), and nutrient water treatments as its reuse leads to easy spread of disease (Savvas et al., 2013). Hydroponic systems can be further divided depending upon the method (sub irrigation, drip irrigation, stagnant) used to deliver the nutrient feed water to plants roots, and the type of substrate or container used to hold the plants (Maucieri et al., 2018).
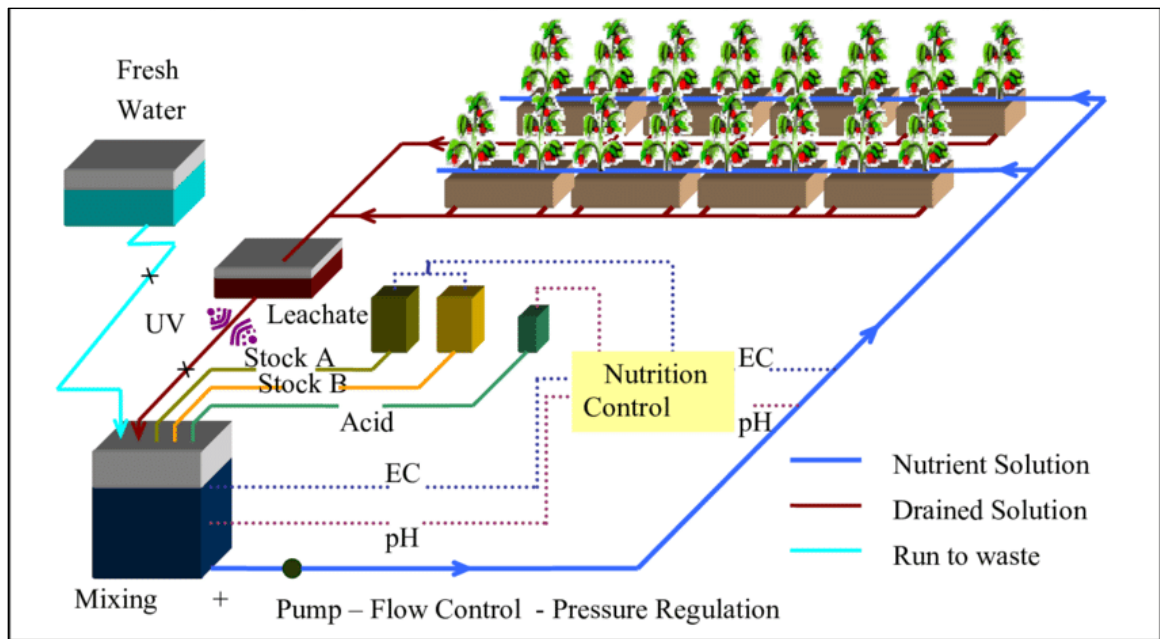
Figure 3: Closed hydroponic system where nutrient water is recovered and recirculated (Source:

Savvas et al., 2013).
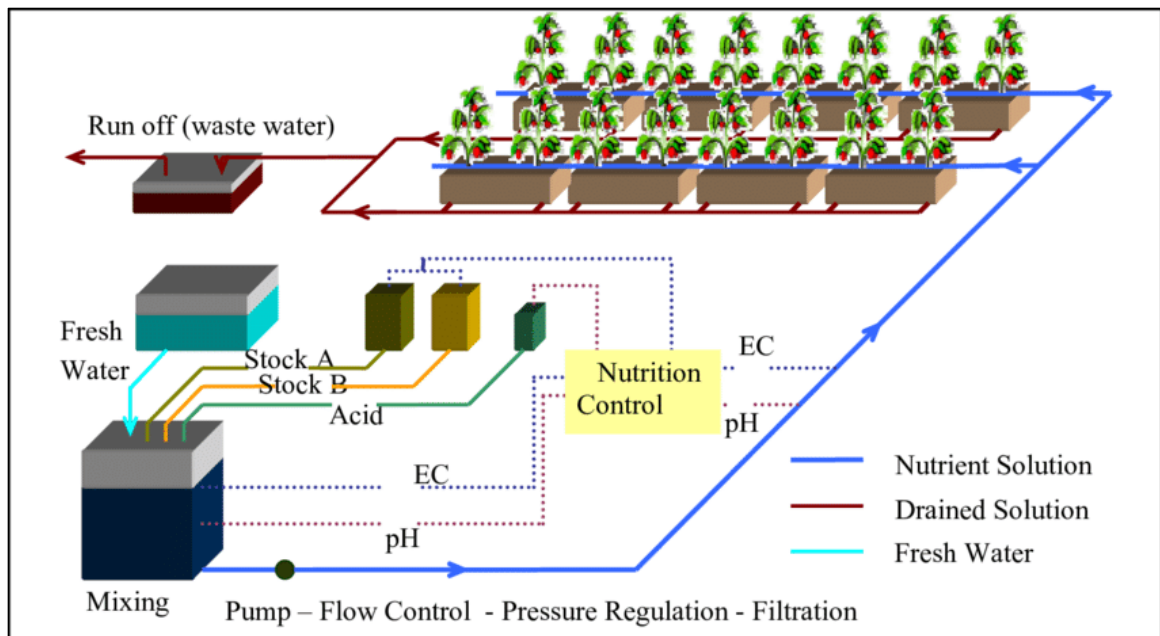
Figure 4: Open hydroponic system where nutrient water is not recirculated but is discarded

(Source: Savvas et al., 2013).

1.1.2.2.1 Standing aerated nutrient solution/ floating systems

In this method, a Styrofoam board/base is used for plant support (Tajudeen and Taiwo, 2018) which floats above the nutrient water. The roots are suspended in a tray filled with nutrient water which is aerated continuously. This results in frequent stirring of the nutrient water. In this system, it is difficult to monitor and maintain the pH and electrical conductivity (EC) of the water as it is constantly changing. Also, the water and fertilizer use are high. For these reasons, standing aerated nutrient solution method has very limited commercial use. On a smaller scale, it is used for cultivation of leafy greens and herbs like basil (Savvas et al., 2013).

1.1.2.2.2 Ebb and flow method

Ebb and flow systems are also called flood and drain systems. It consists of three basic parts- a growing chamber with a light source, a reservoir tank, and a submersible pump (Singh et al., 2017). The method involves pumping the nutrient solution from a holding tank and flooding the holding table periodically, allowing it to drain into same holding tank then recirculating the nutrient water (Rorabaugh et al., 2002).

1.1.2.2.3 Nutrient film/ flow technique (NFT)

NFT was the first hydroponic technique used on a commercial level in the UK. This technique is used for small and quick growing produce like lettuce (Dholwani et al., 2018). This is

a closed system in which the plant roots are suspended in a long channel made of a flexible

material with a slope such that the nutrient feed water can flow under gravity. At the lower end,

the nutrient solution is collected and recirculated (Kaan, 2008). The disadvantage of this method

is, there is no proper mixing of the feed water, as the water flows from top to bottom of the

channel, it encounters root masses leading to reduction in amount oxygen and nutrients present

in water as it flows down. These root masses are thick and dense which limits nutrient

absorption due to less available surface area (Pardossi et al., 2011). NFT involves no use of

substrate, reuse of nutrient solution thus, less quantity of water is needed to run the system

making NFT one of the most efficient hydroponic method for cultivation of crops (Savvas et al.,

2013).

Table 4: Characteristics of different hydroponic systems

Characteristic	Substrate and drip irrigation/ebb and flow	NFT	Floating systems
Commercial use	Large	Limited	Large
Crops grown	Tomato, cucumber, strawberry	Leafy vegetables	Flowers, leafy vegetables Not suitable for fruit vegetables

Substrate	Yes	No	Yes
Nutrient solution recirculation	Yes/no	Yes	Stagnant water
Root aeration	Moderate	Moderate	Low to moderate
Initial investment	Moderate/ high	High	Low
Operating cost	Low	Moderate	Low
Reference	Pardossi et al. (2011)	Stanghellini and Rasmussen (1994)	Hasan et al. (2018); Savvas and Gruda (2018)

1.1.2.3 Hydroponic substrates

There are different types of organic and inorganic materials that are used to provide mechanical support to the plants in hydroponic systems (Stanghellini and Rasmussen, 1994). These are called substrates or growing mediums. The growing medium provides aeration, support to the roots, and aids in their growth (Olle et al., 2012).

A good substrate material must have high porosity (50-85%), low bulk density, low soluble salt content, and must be free of any pathogens (Pardossi et al., 2011). Coconut fiber is an example of organic material that is used as a substrate. Rockwool, which is one of the most common support materials is an inorganic material (Giurgiu et al., 2014; Stanghellini and Rasmussen, 1994). Wide use of rockwool in greenhouse cultivation of crops is due to its easy availability, low cost, ease of handling, and excellent aid in plant growth (Savvas, 2003; Savvas and Gruda, 2018). It is a man-made fiber synthesized by melting volcanic rock and limestone mixture, and spinning it into fibers (Dowgert, 2013). It is free of pathogens because of use of high temperature (1600 °C) involved in its manufacturing (Savvas, 2003). When soaked in a nutrient solution, it holds 80% nutrient solution, 15% air, and 5% of rockwool fibers. This ratio of solution to air is desirable for healthy plant root growth (Dowgert, 2013; Putra and Yulinado, 2003).

Table 5: Substrate characteristics for most commonly used materials in hydroponic systems

Substrates	Characteristics	Bulk density (kg/m^3)	Total porosity (%v/v)	Reference
Rockwool	Lightweight, clean, non-toxic, inert, good aeration, and water holding capacity, provides good plant growth	80-90	94-97	Giurgiu et al. (2014); Hasan et al. (2018); Putra and Yulinado (2003)

Vermiculite	Lightweight, porous, high water absorption capacity	90-150	90-95	Hasan et al. (2018)

1.1.2.4 Plant root system in hydroponics

The species and physical environment of the plant affects its root structure. Two main functions of roots are to provide physical support to the plant, and absorb water and nutrients. Healthy roots appear white in color. As plants grow slight yellowing can be observed (Roberto, 2003). Root characteristics such as color, length, degree of branching, presence of root hair are affected by the hydroponic system environment. In hydroponic system, roots take up more growing area by volume as compared to in soil. Also, root hairs are absent (Jones, 2014; Rorabaugh et al., 2002). Carbohydrates required for root growth are provided by the plant shoots. It is sufficient to have one functional root in hydroponic growing system as its function is only to take up nutrient water hence extensive network of roots can be detrimental to plant growth as they require continuous carbohydrate supply. For well-functioning roots, they are kept well aerated and at a temperature between 20 °C - 30 °C. Root physiology is affected by nutrient solution characteristics such as form of nutrients present, source of nutrients, and interactions among them (Sambo et al., 2019).

1.1.2.5 Plant nutrition

There are 16 mineral elements that are necessary for healthy plant growth. The nine major elements or macronutrients include C, H, O, N, P, K, Mg, S, and Ca. While C, H and O are

obtained from carbon dioxide and water (Dzida et al., 2013) the other minerals must be absorbed from a soil or provided to the plant. Carbon is also absorbed by the roots in the form of HCO_3^-. Oxygen is taken up by the roots in gaseous form and dissolved form (Trejo-Téllez and Gómez-Merino, 2012). All remaining elements have to be provided via the nutrient solution. The nine macronutrients make up to 95% of the dry weight of the plants. Whereas micronutrients such as elemental Mn, Fe, B, Cl, Cu, Mo, Zn which are required in smaller quantities, constitute to less than 0.01% of the dry matter. Among all the macro and micronutrients, nitrogen is one of the most important nutrient responsible for quality and yield of plants (Dzida et al., 2013).

Table 6: List of all essential nutrients, their function, and recommended range for hydroponically grown plants

(Source: Khan et al., 2018; Rorabaugh et al., 2002; Jones, 2014; Silber and Bar-Tal, 2008; Zheljazkov et al., 2008 a)

Nutrient	Function	Utilized forms	Deficiency	Excess	Recommended range (ppm)
C	Basic building block of cells	-	Reduced growth	-	
O	Cellular respiration	Derived from water molecule, ionic forms	Browning of roots, tissue death	-	

		of nutrients			
H	Nutrient cation exchange	Derived from water molecule, ionic forms of nutrients	-	-	
N	Chlorophyll and protein synthesis	NO_3^-, NH_4^+	Light colored leaves and yellow in older leaves, stunted growth	Dark green leaves, restricted root system	100-200
S	Component of plant protein	SO_4^{2-}, SO_2	Not common, Reduced nitrogen uptake, light colored leaves	Premature senescence	50-100
K	Enzyme activity, membrane permeability control,	K^+	Scorching in leaves	Magnesium deficiency	100-150

	osmotic balance				
Mg	Enzyme activation, chlorophyll component, membrane permeability control	Mg^{2+}	Chlorosis and yellowing of leaves	Brown tips in roots and leaves	50-80
Cu	Enzyme activation	Cu^{2+}	Slow plant growth, distorted leaves	Stunted roots, iron deficiency	
Zn	Enzyme component	Zn^{2+}	Small chlorotic distorted leaves	Iron deficiency	0.1-0.5
Mo	Enzyme activators, nitrogen metabolism	Mo_4^{2-}	Light colored leaves and yellow in older leaves, stunted growth	Very rare	0.05-0.15

P	Root growth	Phosphates (HPO_4^{2-}, $H_2PO_4^-$)	Stunted growth, accumulation of anthocyanin in older leaves, delayed plant maturity	Fe and Zn deficiency	15-30
Fe	Energy transfer reactions	Fe^{2+}, Fe^{3+}	Chlorosis	Necrotic spots	0.5-5
Ca	Cell growth	Ca^{2+}	Brown tips in roots and leaves	Not known	150-200
B	Reproduction	Boric acid or borate ion	Abnormal plant development, brittle stems	Brown leaf tips	0.1-0.3
Cl	Root growth	-	Wilting of leaves	Yellowing, reduced growth rate, leaf abscission	-
Mn	Chlorophyll component	-	Chlorosis	Brown spots	0.1-1

1.1.2.6 Hydroponic set up: External factors

1.1.2.6.1 Nutrient solution

One of the advantages of hydroponics over traditional cultivation practices is uniform distribution of nutrients in the solution as compared to soil where there are microclimates present (Nguyen et al., 2016). Nutrient solution supplied to the plants is their only source of getting all the essential nutrients. Most of the nutrient solutions are made from mixing one or all of the following reagents.

1. Calcium nitrate [$Ca(NO_3)_2 \cdot 4H_2O$)]

2. Potassium nitrate (KNO_3)

3. Potassium dihydrogen phosphate (KH_2PO_4)

4. Magnesium sulfate ($MgSO_4 \cdot 7H_2O$)

Nutrient solution is made using nutrient stock solution, water, and acid. The nutrient solution must have adequate balance of all the essential nutrients in their ionic form (Rodríguez et al., 2018). Thus, the inherent quality of water also plays a very important role in supplying adequate nutrition for healthy plant growth.

Table 7: Desirable characteristics of water used to prepare nutrient solution

(Source: Jones, 2014; Hasan et al., 2018)

Characteristics	Desired level
EC	0.2-0.5 µS/cm
pH	5.5-6.8
Alkalinity	40-65 ppm
Hardness	<100 ppm
Na	<50 ppm
Chloride	<90 ppm
Ca	<50-80 ppm
Fe	<0.5-1 ppm
B	<0.3 ppm
Zn	<2 ppm

Electrical conductivity (EC) is the most important nutrient solution characteristic. The effect of EC on plant growth varies according to the plant species. Too low EC can cause inadequate nutrition whereas too high values can expose plants to more saline conditions (Putra and Yulinado, 2003). Savvas (2001) studied the effect of change in EC on growth in tomato

plants. There was linear decrease in the plant yield at EC values above 2.5 mS/cm. This was

attributed to increase in the salinity leading to decrease in the water absorption by the roots.

The maximum fruit yield was obtained at EC values ranging from (2.2-2.5) mS/cm.

The pH of nutrient feed water affects nutrient uptake by the plant roots. The

recommended pH range for the nutrient solution is 5.5-6.5 (Pardossi et al., 2011; Kotzen and

Applebaum, 2016). The pH adjustment is done using acids like phosphoric acid or bases like

potassium hydroxide. Precipitation of nutrients such as Fe, Zn, Cu can take place above pH 7.

Higher pH can also lead to precipitation of macronutrients especially Ca and P (Sambo et al.,

2019).

The pH of the nutrient solution is regulated by the form of nitrogen present. NO_3^-

(nitrate) is alkalizing whereas NH_4^+ (ammonium) is acidifying. Plants primarily use NO_3^- as their

nitrogen source. High concentrations of NH_4^+ can lead to toxicity leading to reduced growth rate,

chlorosis in leaves (Britto and Kronzucker, 2002). Experiments done on tomato showed stunted

root development because of high concentration of NH_4^+ (Sambo et al., 2019). With 3:1 ratio of

NO_3^- to NH_4^+, optimal root growth with maximum surface area and volume was observed in

tomato plants (Sambo et al., 2019; Na et al., 2014). Canola plants grown on 1:1 NO_3^- to NH_4^+ ratio

in saline feed water showed increased growth (Bybordi et al., 2012). This shows that the

susceptibility of plant growth towards nitrogen form depends on the species and plant

environment (Zhou et al., 2011 a; Sambo et al., 2019). According to Adler and Simon (1989),

nitrogen form affects essential oil composition and content in basil plants. They studied the

effect of NH_4^+ and NO_3^- as a sole nitrogen source for sweet basil and found that NH_4^+ as a sole

source of nitrogen caused 28 % reduction in the essential oil content. Appropriate balance of

these two forms is crucial in maintaining the desirable pH and providing adequate nutrition to growing plants (Jones, 2014).

For a hydroponic set up, the temperature of the nutrient solution must be same as that of the room temperature. The temperature higher or lower than room temperature can result in improper water and nutrient uptake by the roots. Temperature affects dissolved oxygen content in the nutrient solution. With an increase in temperature the dissolved oxygen content reduces (Trejo-Téllez and Gómez-Merino, 2012). Urrestarazu et al. (2005) showed that dissolved oxygen level of <3 ppm led to root browning and stunting in hydroponic tomato and cucumber plants. Also, any changes in temperature of the nutrient feed solution can cause precipitation, complexation reactions leading to altered form of some nutrients in the solution changing the nutrient availability for root absorption (Sambo et al., 2019).

It has been shown that autotoxicity can be caused by release of certain compounds from plant roots. Benzoic and salicylic acid adversely hampered the growth of fava beans and strawberry roots and shoots (Asaduzzaman and Asao, 2012; Kitazawa et al., 2005). Thus, it is recommended to recirculate the nutrient solution for no more than 2-3 weeks (Jones, 2014).

1.1.2.6.2 Light conditions

Light is one of the most important factors influencing plant growth. The light duration, quantity, quality, and direction all have significant effect on the plant growth. Light intensity is measured as photosynthetic photon flux density (PPFD given in $\mu mol/m^2s$) and is a standard way of quantifying light. PPFD is measured using light meters. DLI (daily light integral given in mol/m^2d) is also used to quantify light. DLI is product of PPFD and photoperiod. Optimum

exposure to daylight hours is called photoperiods which varies according to the plant species. For basil, long photoperiods are recommended due to its tropical origin (Dou et al., 2018).

Red light (wavelength between 600 nm - 680 nm) and blue light (wavelength between 380 nm - 480 nm) are responsible for photosynthesis. Commercially, high intensity discharge lighting is used for indoor plant cultivation of which two main types are- metal halide lamps which emit more of blue light, ideal for leafy crops like lettuce, and high-pressure sodium lamps which emit more of red light, ideal for flowering and fruiting crops. There combination also can be used for indoor plant cultivation (Roberto, 2003).

Artificial lighting accounts for 80% of the energy used in indoor cultivation of plants (Dou et al., 2018). Generally, 10 h to 16 h photoperiod is recommended for indoor cultivation of plants (Roberto, 2003). Effect of 10 h and 16 h photoperiods on caraway and dill plants was studied by Putievsky (1983 a). They found significant morphological differences in terms of number of branches, plant height, root weight, and onset of flowering. Caraway needed short photoperiods whereas dill was favored by long photoperiods. In another study, Putievsky (1983 b) found that day length (10 h and 16 h) did not affect the plant height in basil and oregano plants but caused an increase in the fresh weight for both. Ren et al. (2014) studied the effect of LED lighting on phenol concentration in *Gynura bicolor DC* known as Okinawan spinach. They found that 80 % red and 20 % blue light led to increased phenol production as compared to 60 % red and 40 % blue light. Amaki et al. (2011) studied the effect of combined white (white, blue, green, and red in the ratio 3:1:1:1, respectively), blue (470 nm), green (525 nm), and red (660 nm) LED lights on growth and essential oil content of basil. Total leaf weight and leaf area was highest under green light. Leaf weight and area of lateral shoots was higher under blue light, and essential oil content was significantly higher than combined white, red and, green light.

1.1.2.6.3 Temperature and relative humidity

For efficient use of CO_2 and light, temperature in the green house must be maintained at the optimum level according to the plant species (Hückstädt, 2013). Use of lamps for light generates heat so frequent ventilation is necessary to maintain the indoor temperature between 18 °C - 24 °C (Roberto, 2003). Increasing the day temperature from 28 °C to 32 °C led to significant increase in the fresh weight of basil and in contrast for oregano there was a decrease in the fresh weight (Putievsky, 1983 b). For different tomato varieties such as *Solanumlycopersicum* "Mecano", "Capricia", "Cederico", changing the temperature from 23 °C to 29 °C showed no effect on its dry weight (Hückstädt, 2013).

Air relative humidity changes according to change in the temperature, transpiration and condensation process. Evapotranspiration is caused by low relative humidity. High relative humidity promotes mold growth, increased water condensation, and low transpiration rate leading to physiological disorders (Hückstädt, 2013). Most crops benefit with relative humidity levels between 60 % - 70 % (Roberto, 2003).

1.1.2.6.4 Carbon dioxide

Elevated CO_2 level can enhance photosynthetic processes in plants leading to more growth and yield. For indoor cultivation of vegetables, CO_2 is used as a gas fertilizer in the range of 700 ppm - 1000 ppm but not much research is done on the quality of such produce (Dong et

al., 2018). It has been reported in the literature that higher CO_2 concentrations up to 900 ppm led to marked increase in the tomato fruit yield (Yelle et al., 1990).

1.1.2.7 Diseases of hydroponically grown plants

Most of the pests and pathogens are soil borne (Asaduzzaman et al., 2013). Hydroponics was adopted in commercial cultivation of produce to give more control over soil borne pathogens (Chinta et al., 2015). Use of hydroponics has reduced the diversity of root infecting pathogens as compared to traditional soil-based cultivation. However, root infecting pathogens are still a major cause of yield loss in hydroponics. This is especially a problem in closed hydroponic systems where nutrient water is reused (Stanghellini and Rasmussen, 1994). Bacterial infection is very rare in hydroponic set up (Schnitzler, 2003). Most problems arise from fungal infections.

The fungus, *Botrytis cinereal,* is the most destructive air borne pathogen causing gray mold in hydroponic crops. Chinta et al. (2015) studied the effect of organic and chemical hydroponics on gray mold symptoms and found that organically produced lettuce showed much less severe symptoms. Additional fungal diseases include *Pythium spp., Phytophthora spp.*, and *Plasmopara spp.* cause the most damage to produce in a recirculating hydroponic system (Schuerger and Hammer, 2009; Stanghellini and Rasmussen, 1994). These organisms can get introduced to nutrient solution from water, seed, insects, dead plants, footwear, and machinery used. Oospores of fungi *Pythium* can survive for a long time and can lead to infecting subsequent batches of produce. In hydroponic systems, nutrient solution is not as diverse in terms of microbial population as the soil. This creates weak biological resistance to *Pythium* in

hydroponic produce (Sutton, 2007). Hydroponically grown endive and fennel in a recirculating gravel bed were affected by *Pythium F* group resulting in stunted growth and root rot (Labuschagne et al., 2003). McGehee et al. (2018) reported incidence of root rot in hydroponic lettuce grown in deep water culture system caused by *Pythium dissotocum* resulting in leaf necrosis and death. Herrero et al. (2008), reported the first presence of root rot caused by *Phytophthora capsici* on hydroponically grown cucumbers using rockwool as a substrate resulting in 10 % plant death.

Egel et al. (2010) have reported the occurrence of black leg disease in hydroponically grown basil. Sweet basil plants showed dark legions on the stem just above the nutrient water interface resulting in brittle unmarketable produce. This was caused by fungus *Plectosporium tabacinum*. Sweet basil grown in pots containing Fafard potting medium in a greenhouse developed leaf necrosis caused by *Plectosphaerella Cucumerina* (Mersha et al., 2012).

The common disease control methods in hydroponics include use of resistant cultivars, improved sanitation, use of fungicides, nutrient solution treatment, and manipulation of greenhouse environment (Stanghellini and Rasmussen, 1994; Roberto, 2003).

1.1.2.7.1 Algae growth

Growth of algae in hydroponic set up is favored due to the presence of abundant light and nutrients. Algae competes with growing plants for nutrients, oxygen, and water and thus strategies such as limiting light exposure of roots, using open system, keeping nutrient solution reservoir away from light are employed (Roberto, 2003; Somerville et al., 2014).

Chlamadomonas spp. is the most commonly found algae in a closed hydroponic system. It limits the growth of other algae species such as *Klebsomidium spp.* and *Chlorella vulgaris* (Schwarz and Krienitz, 2005). Barone et al. (2019) studied the effect of co-cultivation of microalgae *Chlorella vulgaris* and *Scenedesmus quadricauda* with tomato plants by adding microalgae suspension to nutrient solution and found that co cultivation had a positive effect on microalgae growth as well as tomato plants growth due to secretion of bio stimulating substances by the microalgae.

1.1.2.8 Product quality and consumer acceptance

With use of hydroponics, it is possible to get more yield/unit area due to efficient use of available space and continuous production. However, it is important to understand the technical know-how in terms of maintaining optimum nutrition and environmental conditions for specific produce.

There was no significant difference in quality of lettuce (Frezza et al., 2005), melon (Guler et al., 1993), spring onion (Thompson et al., 2005) grown using soil and hydroponic methods. Treftz and Omaye (2015) observed more yield, less plant death, and less variability in fruit size in strawberries grown using hydroponic method as compared to open field cultivation.

Synthesis of sugars, acids which are responsible for sensory quality, and secondary metabolites can be manipulated by having more control over the produce environment (Gruda, 2009). Palermo et al. (2011) reported more fat and dietary fiber content in soybeans grown

hydroponically as compared to soybeans grown on soil resulting in their improved quality. Increase in EC by increasing nutrient concentration caused significant increase in total solids, vitamin C, lycopene, and beta carotene in fresh tomato (Krauss et al., 2006), increased mineral content in celery (Pardossi et al., 1999), and in cucumber (Trajkova et al., 2006). Rouphael et al. (2004) reported more concentration of glucose, fructose, and starch in hydroponically grown zucchini. Sgherri et al. (2010) analyzed the antioxidant activity of (*Ocimum basilicum* cv. 'Genova') basil extracts and found that hydroponically grown basil showed significantly more antioxidant activity due to increase in the content of vitamin C, total phenols, vitamin E, and rosmarinic acid. Buchanan and Omaye (2013) studied ascorbic acid and tocopherol content in three lettuce varieties and reported more than two-fold increase in all hydroponically grown varieties compared to their soil grown counterparts.

Due to lack of awareness, consumers tend to think soilless agriculture uses more chemicals as compared to traditional practices which provide nutrients to plants from soil (Savvas, 2003; Gruda, 2009). In organic farming, all the nutrients come from an organic source (Savvas et al., 2013). However, plants absorb most of the nutrients in inorganic form, they absorb nitrogen in the form of NO_3^- irrespective of the source it came from (Jones, 2014; Khan et al., 2018). The source of nutrients, organic or inorganic does not affect the quality of the produce but the amount of the nutrient absorbed does (Savvas, 2003). Murphy et al. (2011) compared sensory qualities such as taste, color, appearance, and texture using descriptive analysis on 5-point scale between commercially available five organic lettuce varieties and hydroponically grown varieties. They found that all five organic and hydroponically grown varieties were rated equally on all sensory qualities.

Thus, using hydroponics, which is a soilless agriculture technique, it is possible to produce higher crop yields with better quality in terms of appearance, taste, and bioactive compound content.

1.2 Basil

1.2.1 Origin, history and classification

The word basil is derived from Greek word *basilikos* meaning royal. Out of all the basil species *Ocimum basilicum* L. (sweet basil) is most widely cultivated and used (Razavi and Naji-Tabasi, 2017). *Ocimum* originates from *okimon,* a greek word meaning smell. Basil is famous for its rich, spicy and peppery flavor. In Hindi, basil is called *tulsi*, *basilico* in Italian, *basilica* in French, and *rehan* in Arabic. It is said that basil was introduced to Greece by Alexander the great (350-320 BCE). In 1500s, through the spice trade, basil was brought to England (Lupton et al., 2016). There are some interesting beliefs associated with basil. In India, Hindus use holy basil leaves in burial rituals for the safe passage of the dead to heaven (Makri and Kintzios, 2008; Meyers, 2003).

Although basil originated in Asia, it is now widely cultivated in many countries such as Egypt, France, Greece, Hungary, and Morocco. In the US, basil is widely grown in California, North Carolina, and Arizona (Dhar, 2002; Pushpagadan and George, 2012). The center of diversity of basil is in Africa and secondary centers are in India and Brazil (Vieira et al., 2003). Sweet and holy basil are two most widely grown species of basil in the world (Zheljazkov et al., 2008 b). In the US, basil is grown on more than 5000 ha every year (Homa et al., 2016).

Basil has been cultivated for centuries which has resulted in great diversity in terms of its morphological characteristics such as height, color of the plant, size and shape of the leaves. However, there are challenges in the classification of basil species due to interspecific hybridization and polyploidy within the genus (Simon et al., 1990). Also, species which differ in their essential oil profile (chemotype) might not show same morphological characteristics (Vieira et al., 2003). The essential oil profile is based on type of cultivar, hybridization, process of extraction and storage (Chowdhuri et al., 2016). Pushpangadan and Bradu (1995) have reported more than 150 species of genus *Ocimum*. According to Paton (1999), there are 65 species and the rest can be called synonyms. Due to the promiscuous nature of basil, there is a disagreement in researchers about its total number of species (Meyers, 2003). Thus, classification using only morphological descriptors or essential oil profile is not accurate. Use of morphological characteristics and essential oil profile along with molecular markers will result in better classification among the genus (Chowdhuri et al., 2016).

Basil taxonomic classification (USDA, 2020)

- Kingdom: *Plantae* – plants

- Sub-kingdom: *Tracheobionta* – vascular plants

- Superdivision: *Spermatophyta* – seed plants

- Division: *Magnoliophyta* – flowering plants

- Class: *Magnoliopsida* – dicotyledons

- Sub-class: *Asteridae*

- Order: *Lamiales*

- Family: *Lamiaceae* – mint family

- Genus: *Ocimum* L. – basil

- Species *Ocimum basilicum* L. – sweet basil

Table 8: Classification of basil according to its chemotype

Chemotype	Origin	Characteristic compounds	Reference
European	Italy, France, Egypt, South Africa	Linalool, Methyl chavicol	Telci et al. (2006)
Tropical	India, Pakistan, Guatemala, Haiti	Methyl cinnamate	Vernin and Metzger (1984); Telci et al. (2006)
Reunion	Thailand, Madagascar, Vietnam, Comoro island	Methyl chavicol	Simon et al. (1990)
Eugenol rich	North Africa, Russia, Eastern Europe, Parts of Asia	Eugenol	Vernin and Metzger (1984); Zheljazkov et al. (2008 a)

Table 9: Basil classification according to morphology (Dhar, 2002)

Type	Approximate plant height
Tall and slender	90 cm

Large leaf	75 cm
Dwarf, small leaf	<30 cm
Compact	Variable

1.2.2 Morphology

Basil plant/leaves have wide range of form, color (green and purple), and size. Plants are shrubby and can grow up to 3 m tall. Leaf texture depends on species and can vary from smooth, shiny to hairy, and curly. The leaves contain many oil glands. In purple and green large leaf varieties, the leaf edge is serrated and it is non-serrated in small leaf varieties (Lupton et al., 2016; Bilal et al., 2012). The petiole (stalk attaching a leaf to plant stem) is (1.3-2.5) cm long (Bilal et al., 2012). Flowers are white to purple (Meyers, 2003). Flowering can be seen after 19-60 days of planting (Svecova and Neugebauerova, 2010). Basil leaves, flowers, and seeds are edible (Makri and Kintzios, 2008). Seeds get matured after flowering resulting in no further growth of new leaves (Meyers, 2003). Basil seeds are oval and black, and are rich in fiber (22.6%). They are usually (3.22 ± 0.33) mm in length, (1.84 ± 0.24) mm in width, and (1.37 ± 0.24) mm in height (Razavi and Naji-Tabasi, 2017). Seeds become mucilaginous in contact with water because of their high fiber content (Dhar, 2002).

1.2.3 Ecology

Basil is an annual herb and perennial varieties such as *O. tenuiflorum* can be found in tropical regions (Tilebeni, 2011). Globally, basil is cultivated under wide range of environmental parameters- 7 °C to 27 °C, 0.6 m to 4.2 m annual precipitation, and 4.3 to 8.2 soil pH (Simon, 1995). Basil grows well in regions of warm temperatures and high rainfall. Such conditions are also deemed favorable for higher oil synthesis (Pushpagadan and George, 2012). The optimum rainfall, soil pH, and atmospheric temperature are 1.3 m/year, 6.4, and 25 °C, respectively, (Meyers, 2003; Chang et al., 2005).

1.2.4 Common uses

Basil plant leaves, stem, flower, and seeds have many uses in medicine and culinary science around the world (Dzoyem et al., 2017). Basil has been reported useful in treatment of more than 100 conditions (Meyers, 2003). Nowadays basil is grown as an ornamental plant in home gardens. Purple leaf varieties are used for decorative purposes (Svecova and Neugebauerova, 2010). Essential oil extracted by steam distillation is used in dental hygiene products (Simon et al., 1990).

1.2.4.1 Culinary uses

Basil is used as a flavoring agent in many cuisines. Small leaves are used whole and larger leaves are chopped. Basil flavor goes very well with cheese, vinegar, oil, and tea (Meyers, 2003). Basil is used to flavor spicy meats, ice creams, confectionary products, etc. It is most commonly used to flavor tomato-based food preparations in Italian cuisine (Pushpangadan and George, 2012). Soaked basil seeds are used in many Indian desserts like Falooda (Razavi and Naji-Tabasi, 2017).

1.2.4.2 Therapeutic uses

Basil is used as a medicinal herb to treat common ailments such as cough, diarrhea, worms (Telci et al., 2006). In folk medicine in Asia, aerial parts of basil are used for their carminative, stomachic, and antispasmodic properties (Sajjadi, 2006). Basil oil is used to treat spasms and mental fatigue (Bilal et al., 2012). It is also used to treat insect bites and snake bites (Purushothaman et al, 2018; Bilal et al., 2012). Basil juice and honey mixture is used to treat cold and cough symptoms.

1.2.5 Nutrition

Table 10: Nutrient content of 100 g of fresh and dry basil (USDA, 2019)

Nutrient	Fresh	Dry
Water	92.06 g	10.35 g
Energy	23 kcal	233 kcal
Protein	3.15 g	22.98 g
Fat	0.64 g	4.07 g
Ash	1.49 g	14.85 g
Fiber	1.6 g	21.7 g
Sugars	1.71 g	17.1 g
Ca	177 mg	2240 mg
Fe	3.17 mg	59.9 mg
Mg	64 mg	711 mg
K	295 mg	2630 mg
Vitamin C	18 mg	0.8 mg
Folate	68 µg	310 µg

Vitamin A	5275 IU	744 IU
Vitamin E	0.8 mg	10.7 mg
Vitamin K	414.8 µg	1714.5 µg

1.2.6 Chemistry of basil essential oils

Distinct flavors of basil are due to their essential oil composition. Basil essential oil is a mixture of phenols, monoterpenes, and sesquiterpenes (Dhar, 2002). It is made of three main components which are phenol derivatives- linalool, methyl chavicol, and eugenol (Meyers, 2003; Purushothaman et al., 2018; Makri and Kintzios, 2008). Their percentage varies depending on the basil variety. Methyl chavicol gives sweet taste. It is found prominently in sweet varieties. In spicy varieties, more amount of eugenol is found which gives of clove like flavor (Meyers, 2003). Basil leaves also contain flavonoids such as quercetin, isoquercetin, rutin, esculin, and caffeic acid (Dhar, 2002).

1.2.7 Cultivars

More than 40 cultivars of basil are commercially available (Simon et al., 1999). Popular basil cultivars include *O. basilicum* 'Cinnamon', *O. basilicum* 'Dark Opal', and holy basil (the species *O. tenuiflorum* L., previously known as *O. sanctum* L.) (Lupton et al., 2016).

Table 11: Commercially important cultivars of *O. basilicum*

Cultivar	Characteristic	Flavor	Reference
O. basilicum 'Genovese'	Dark green leaves, up to 2 in long, best use for pesto	Sweet and peppery, minty	Makri and Kintzios (2008)
O. basilicum 'Crispum'	Curled leaves	Sweet, sometimes anise	Darrah (1974)
O. basilicum 'Lactucaefolium' (Italian basil)	Broad leaves	Sweet	Simon et al. (1999)
O. basilicum 'Purpurascens'	Purple leaves	Sweet	Darrah (1980)
O. basilicum 'Dark opal'	Lobed purple leaves	Sweet and clove	Simon et al. (1999)
O. basilicum 'Citrodorum'	-	Orange blossom	Darrah (1974)
O. basilicum 'Minimum'	Small leaves	Pungent and bitter	Darrah (1974); Simon et al. (1999)

1.2.8 Production practices

Commercially available basil is of Genovese type and is characterized by its large leaf size (Pyne et al., 2014). In the US, basil production is done mainly in fields and greenhouses. In the recent years, basil production using hydroponic systems such as nutrient film technique (NFT) is on the rise. While the demand for fresh basil has increased greatly, its year-round production and supply is possible because of controlled environment agriculture where proper temperature, relative humidity, and light conditions are maintained (Walters and Currey, 2015).

Soil cultivation of basil is done using direct sowing or nursery sowing. Harvest can be done 3-5 times a year. Usually the first harvest is done after 75-90 days (Dhar, 2002; Pushpangadan and George, 2012). It is recommended to harvest basil during morning hours due to its strong essential oil activity (Lupton, 2016; Da-Silva et al., 2003; Meyers, 2003; Carvalho Filho et al., 2006). But for fresh market use, basil is harvested in the evening hours. Basil harvested in the evening hours tends to have longer shelf life. Aharoni et al. (2010) have reported that basil harvested at 4 PM and stored at 12 °C for 5 days showed 42 % less leaf decay and no leaf browning as compared to 8 AM harvest.

1.2.9 Post harvest technology

Post-harvest practices such as handling, transportation, and storage play important role in maintaining the quality of fresh herbs (Satpute et al., 2019). Traditionally, herbs are used in different forms such as fresh, chopped, dried, and powdered. Use of post-harvest technology

depends on the intended use and form of the herb. Aromatic herbs like basil, coriander, parsley are minimally processed for their fresh market use to maintain their color, flavor, and freshness (Santos et al., 2014).

Drying is a reliable method to extend shelf life of herbs, by prolonging onset of biochemical reactions and improving their microbiological quality (Yousif et al., 1999). Researchers have studied different methods such as ambient air drying, microwave drying, sun drying, vacuum drying, and freeze drying to understand the effect of drying method on basil essential oil profile. Due to volatile nature of essential oils, shade drying is preferred as compared to sun drying technique (Lupton et al., 2016). Díaz-Maroto et al. (2004) reported significantly less decrease in the volatiles by using ambient air drying (13.6 %) as compared to freeze drying (27.4 %) and oven drying at 45 °C (28.6 %). Ambient air drying also resulted in better sensory profile of the dried basil. But ambient air drying is commercially not viable as it requires long drying times and gives less control over the process. Pirbalouti et al. (2013) reported that oven drying at 40 °C and freeze drying gave better quality product in terms of cell structure, color retention, and essential oil yield as compared to microwave drying for 4 min at 500 W and direct sun drying at temperatures between 20 °C - 37 °C. Yousif et al. (1999) studied air drying and vacuum microwave drying and found that vacuum microwave drying showed better results in terms of cell structure damage, rehydration capacity, and color. Currently, convective drying and vacuum microwave drying methods are used on commercial basis. Calin-Sanchez et al. (2012) have suggested use of combination of convective pre drying and microwave vacuum finish drying to reduce the cost of drying process and achieve better quality product.

1.2.10 Storage

Shelf life of fresh basil leaves is very short. Fresh basil is best kept by wrapping it in 2-3 layers of paper and storing in airtight container at ambient temperature (Meyers, 2003; Lupton et al., 2016). For longer usage drying is recommended. Dried basil stored in a glass jar can be used up to one year. For home use, traditionally basil leaves are soaked in olive oil and salt in a glass jar and used for up to two weeks (Lupton et al., 2016).

Due to tropical origin of basil, it is prone to chilling injury during transportation and storage. Chilling injury results in leaf browning, necrosis, and wilting (Ribeiro and Simon, 2007). Mature leaves are more susceptible to chilling injury than young leaves. Post-harvest chill hardening is a reliable method to reduce instance of chilling injury in greenhouse grown packaged basil and can aid in its transportation. Post-harvest chill hardening done by exposing the packed basil to 10 °C for one day and then storing at 5 °C, delayed the onset of chilling injury by five days (Lange and Cameron, 1997). The extent of the injury depends on storage time and length. Lange and Cameron, (1994) studied the shelf life of basil at six different storage temperatures (0, 5, 10, 15, 20, 25) °C and based on visual appearance they reported that maximum shelf life of 12 days was observed at 15 °C without any signs of chilling injury. Below and above 15 °C, the shelf life was reduced. According to Aharoni et al. (2010), 12 °C is the optimum storage temperature for basil sold in film packs as it showed no chilling injury however, temperatures higher than 12 °C also showed no instance of chilling injury but led to development of fungal growth.

Modified atmosphere is shown to improve keeping quality of basil. Basil stored for 20 days in modified atmosphere of 2 % oxygen in nitrogen showed better visual quality than

storage using air (Amodio et al., 2004). For inexpensive storage, Anderson et al. (2009) have

suggested using microperforated polypropylene bags and storage in presence of light.

1.2.11 Diseases

Worldwide, basil yield is most negatively affected by Fusarium wilt caused by *Fusarium

oxysporum* f. sp. *basilicum* (Gullino et al., 2012). But in the US, Downy mildew is the cause of

most losses in the basil yield (Patel et al., 2014). Downy mildew was first discovered in 2007 in

Florida, and in 2009 it caused close to 100 % losses in the yield in some farms in New Jersey

(Wyenandt et al., 2010).

Table 12: Selected basil plant diseases

Disease	Agent	Remarks	Part affected	Symptoms	Reference
Fusarium wilt	*Fusarium oxysporum* f. sp. *basilicum*	Fungus infects seeds and can sustain in soil for a long time	Leaves, stems	Stunted and wilted plant, stem discoloration, and asymmetric growth	Meyers (2003); Garibaldi et al. (1997)

				followed by necrosis	
Downy mildew	*Peronospora belbahrii*	Caused by infected seeds, wind dispersed spores, warm conditions favor disease spread	Leaves	Initial symptoms resemble nitrogen deficiency, Yellowing of leaves near the middle vein, fuzzy grayish growth on the lower side of the leaf	Tran (2011); Patel et al. (2014)
Bacterial leaf spot	*Pseudomonas cichorii*	Not severe	Leaves and stem	Brown and black spots on the leaves, stem discoloration	Tran (2011)
Gray mold	*Botrytis cinereal*	High humidity and poor	Leaves, stem	Brown to gray fungal growth	Purushothaman et al. (2018);

		air circulation favor the disease spread, just after harvest maximum chance of infection			Garibaldi et al. (1997)
Root rot	*Pythium* and *Phytophthora*	High humidity and poor air circulation favor the disease spread	Whole plant	Sudden leaf wilting, colorless to dark brown rot on the stem, brown colored roots	Meyers (2003); Garibaldi et al. (1997)

These diseases can be prevented by using aerated and well-drained soil, maintaining sanitary conditions, employing crop rotation, and by employing low density planting (Garibaldi et al., 1997; Meyers, 2003).

1.2.12 Essential oils

Vast number of essential oils are produced from different plants every year. In the US, approximately 50,000 tons of essential oils with $ 700 million market value are produced every year. Essential oils comprise of wide range of low molecular weight (less than 500 Da) compounds (Raut and Karuppayil, 2014). Basil is prized for its essential oils, of which more than 100 tons are produced every year (Daniel et al., 2010). Basil flowers and leaves can be used for oil extraction. The essential oil extraction can be done using hydro distillation, steam distillation, super critical fluid extraction, cold maceration, solvent extraction, and microwave extraction (Purushothaman et al., 2018; Pushpagadan and George, 2012). Hydro distillation and steam distillation are common techniques used for extraction of basil essential oil (Raut and Karuppayil, 2014). Three main components of commercially available basil essential oil are linalool, methyl chavicol, and eugenol (Pyne et al., 2014).

The rich and distinctive flavor of basil is due to presence of essential oils mainly in leaves (Meyers, 2003). The oil content in stems is negligible (Dhar, 2002). The USA and European countries such as France and UK are prominent markets for basil essential oils (Lupton et al., 2016). Generally recognized as safe (GRAS) dose for basil essential oil is 0.01 ppm - 50 ppm for oral consumption (Lupton et al., 2016). Basil essential oils are widely used as a flavoring agent, in perfumery, and medicinal industries (Hussain et al., 2008; Telci et al., 2006). Storage period, cultivar, and environment affects the essential oil content of basil. The approximate essential oil output is 27 kg/ha by utilizing the whole plant of sweet basil (Pushpagadan and George, 2012) and (1.8-14.3) mg/kg of essential oil can be obtained from dry basil depending on the cultivar

(Svecova and Neugebauerova, 2010). Rosmarinic acid, chicoric acid, and caftaric acid are the three most abundant phenolic compounds found in most plants including basil. Lee and Scagel (2009) also report presence of chicoric acid and caftaric acid in basil leaves. Chicoric acid has been reported to inhibit HIV-integrase (Charvat et al., 2006).

Hussain et al. (2008) reported that there was significant seasonal variation in essential oil content of basil. They studied the essential oil content of the species indigenous to Pakistan and found that it varied between 0.5-0.8 % according to season with maximum content being produced in winter and lowest production was observed in the summer months. The seasonal variation also affected chemical composition of essential oils resulting in variation ($p < 0.05$) in antimicrobial and antioxidant properties. Da-Silva et al. (2003), studied essential oil content of basil harvested in 2 different months (August and January) at two different time (8 AM and 4 PM) in Brazil. Higher amounts of essential oil were extracted from basil harvested in the morning hours. They found that the harvest month and time both had significant effect on essential oil content and composition. There are some studies suggesting induced water stress causes increased production of essential oils in plants. It also increases variety of compounds in the oil (Omidbaigi et al., 2003). Simon et al. (1992) reported increase in essential oils from (3.1 to 6.2) μl/g of dry leaf weight after applying water stress.

1.2.12.1 Functional properties of basil essential oils

Lot of research is being done on the basil essential oil applications in variety of fields ranging from use of basil oils to inactivate microorganisms to improvement in skin health. Following are some of the prominent fields where application of basil essential oils was studied.

1.2.12.1.1 Antimicrobial

Linalool, which is the most abundant (56.7-60.6 %) component of essential oils in basil, was found to be effective against nine pathogenic microorganisms. *Staphylococcus aureus* and *Bacillus subtilis* were most sensitive with largest inhibition zones (22.2 mm - 24.4 mm), (20.4 mm - 26.1 mm), respectively, and lowest minimum inhibitory concentration values (0.9 mg/ml), (0.8 mg/ml), respectively (Hussain et al., 2008).

1.2.12.1.2 Antioxidant

Hussain et al. (2008) attributed antioxidant activity of basil essential oils to presence of phenolic compounds and reported 80.3-91.2 % reduction in oxidation of linoleic acid due to basil essential oils. High antioxidant activity of purple basil essential oil is due to presence of anthocyanins (Lupton et al., 2016). Purple basil varieties have more than 14 different anthocyanins. 'Dark opal' contains (16.6-18.8) mg/100 g fresh weight of total anthocyanins which has been reported to have antioxidant properties (Makri and Kintzios, 2008).

1.2.12.1.3 Anticarcinogenic

Aruna and Sivaramakrishnan (1992) reported significant decrease in induced squamous cell carcinomas, a type of skin cancer, on use of basil leaf extract (*Ocimum sanctum* Linn) in mouse model. Berić et al. (2008) examined antimutagenic effect of basil essential oil in microbial test. They found that antimutagenic effect was comparable with the use of vitamin E in different *E. coli* strains.

1.2.12.1.4 Hypolipidemic

Hyperlipidemia has direct adverse effect on cardiovascular health. Use of aqueous basil extract significantly reduced plasma (p<0.02) and liver total cholesterol (p<0.05) in hyperlipidemic rat model (Harnafi et al., 2009).

1.2.12.1.5 Hypoglycemic

Inhibition of alpha glucosidase and alpha amylase is linked to controlling type 2 diabetes. El-Beshbishy and Bahashwan (2012), reported use of aqueous basil extract in positively inhibiting activity of alpha glucosidase and pancreatic alpha amylase *in vitro*. Holy basil extract was reported to stimulate release of insulin from beta cells in perfused rat pancreas (Hannah et al., 2006). Agrawal et al. (1996) studied the effect of holy basil leaf extract on post prandial and fasting blood glucose levels in randomized, placebo controlled, single blind trial. They found significant decrease of 15.8 mg/dl (p<0.02) in post prandial glucose level and of 21 mg/dl (p<0.0001) in fasting blood glucose level.

1.2.12.1.6 Skin health

Rasul and Akhtar (2011) evaluated antiaging effect of 3 % concentrated basil extract on topical skin treatment in a single blind study. They found improvement in skin roughness, scaliness, and moisture which was recorded using noninvasive methods.

1.3 Plasma technology

1.3.1 Plasma: Definition and types

Plasma is the fourth state of matter. It is an ionized gas comprising of free electrons and excited atoms. It contains photons (light species) and other constituents (heavy species). Plasma has a zero net charge as it consists of equal number of positive and negative ions (Dey et al., 2016). Plasma is divided into thermal and non-thermal type based on its mechanism of generation (Dey et al., 2016).

Thermal plasma (TP)

These are equilibrium plasmas where the average electron temperature equals average gas temperature. Lightening is an example of naturally occurring thermal plasma. Electric arc discharge and plasma torch are few examples of TP. Thermal plasma generation involves use of high pressure, and high temperature in order of 10^4 K, with production of electrons (Dey et al.,

2016; Surowsky et al., 2015). It has wide industrial applications. But their limited use is due to high energy consumption, and electrode erosion (Karla et al., 2005).

Non thermal plasma (NTP)

In non-equilibrium plasmas there is a significant difference between the average temperature of electrons and that of gas. The electrons temperature is around 10^4 K; however, the generated ions are around room temperature (Surowsky et al., 2015). Northern lights are an example of naturally occurring non thermal plasma. Non thermal plasmas involve use of narrow range of power and are generated at atmospheric pressure and at temperatures between 30 °C – 60 °C (Dey et al., 2016). Dielectric barrier discharge, corona discharge, microwave discharge, and plasma jets are examples of NTP (Park et al., 2013).

For generation of plasma activated water NTP such as pulsed corona discharge, pulse arc, dielectric barrier discharge, and gliding arc discharge are commonly used plasma discharges (Figure 5).
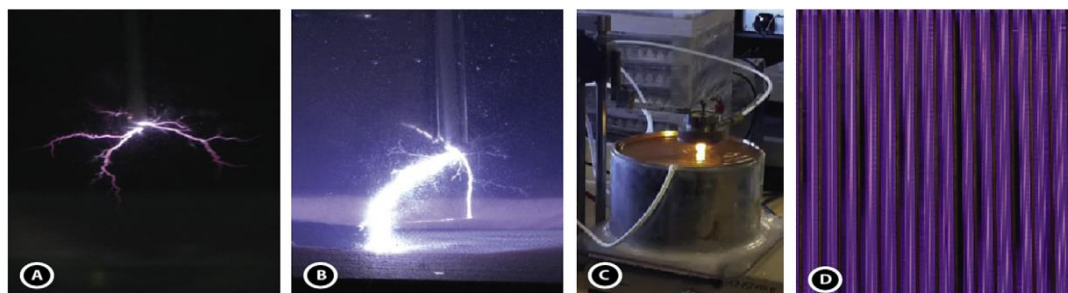
Figure 5: (A) pulsed corona inside water; (B) pulsed arc or spark inside water; (C) gliding arc plasmatron on top of water; (D)dielectric barrier discharge (Source: Park et al., 2013).

1.3.2 Gliding arc plasma

Combination of high level of non-equilibrium and high electron temperature and density cannot be achieved using conventional thermal or nonthermal plasma. Gliding arc combines advantages of thermal and non-thermal plasma.

In this study, gliding arc plasmatron was used to treat nutrient solution. Gliding arc technology generates plasma at atmospheric pressure. It is a type of non-equilibrium plasma (Lie et al., 2006).
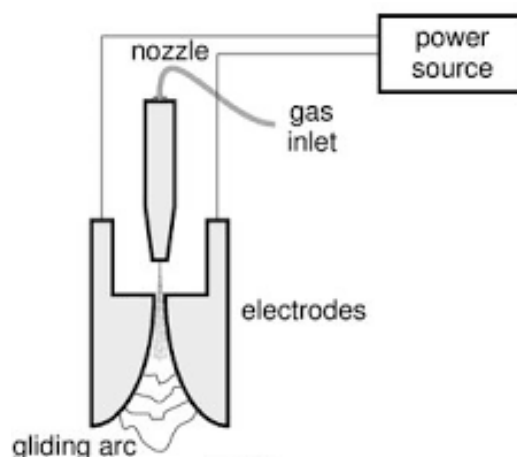
Figure 6: Generation of plasma using gliding arc technology (Source: Sera et al., 2017).

In gliding arc technology (Figure 6), two closely spaced electrodes placed in the air are used to generate plasma. Electric arc is formed at the shortest gap between these electrodes. As the gas flow pushes the arc, it increases in length and forms a plume (Doubla et al., 2007). This creates thermal and non-thermal regions and produces ROS and RNS reactive species. Gliding arc plasma can be generated by two methods (Burlica et al., 2006) which are:

1. Direct exposure- Spraying the liquid through the plasma zone leading to increase in the
   contact surface (Figure 7 a).

2. Indirect exposure- Generating gliding arc above the surface of liquid such that the reactive
   species formed in gas phase get dissolved in the liquid phase and/or are formed at the gas
   liquid interface (Figure 7 b).
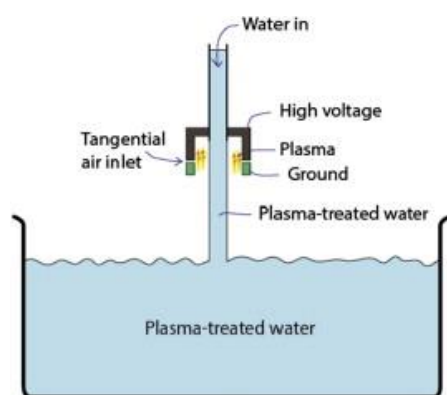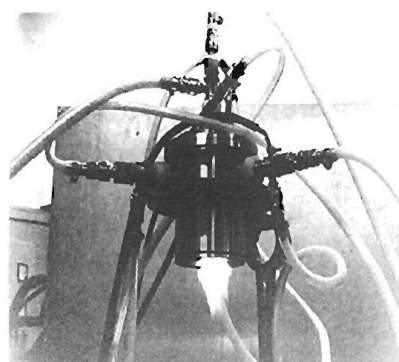
(a)   (b)

Figure 7: (a) Direct exposure gliding arc plasmatron (Source: Park et al., 2013) (b) Indirect
exposure AC gliding arc plasma generator (Source: Lie et al., 2006).

1.3.3 Plasma liquid interaction

Generation of reactive oxygen and nitrogen (RONS) species depends on the type of gas
and liquid used in generating plasma, excited voltage, and generation mode. When air is used as
gas, oxygen, nitrogen, and water are involved in generation of plasma. This creates primary

species such as atomic oxygen, singlet oxygen, superoxide, ozone, hydroxyl radicals, and atomic nitrogen which on further reactions form secondary species such as hydrogen peroxide, peroxynitrite, nitric oxide, nitrates, and nitrite ions. In gliding arc plasma, the most common reactive species formed are superoxide anions, nitrates, and nitrites (Thirumdas et al., 2018).

There are four types of chemical reactions resulting in formation of reactive oxygen (ROS) and reactive nitrogen (RNS) species (Doubla et al., 2007; Surowsky et al., 2015)-

- Acid–base reactions

- Oxidation reactions

- Reduction reactions

- Photochemical reactions

Acid base reactions

Nitrous and nitric acid, nitrates, nitrites, primary species (OH˙ and O˙) and secondary species (H_2O_2, O_3, and ONOOH) contribute to reducing pH of the plasma water. The secondary species formed by plasma and products of primary (OH˙ and O˙) and secondary species (H_2O_2, O_3, and ONOOH) contribute to acidifying effect on water. Apart from this, there is ionization of water due to plasma causing decrease in its pH (Surowsky et al., 2015) as shown by the reactions R1, R2, and R3 below.

$$H_2O + e^- \rightarrow H_2O^+ + 2e^- \qquad\qquad R1$$

$$O^+ + 3H_2O \rightarrow H_3O^+ + 3OH \qquad\qquad \text{R2}$$

$$H_2O + e^- \rightarrow H\cdot + OH\cdot + e^- \qquad\qquad \text{R3}$$
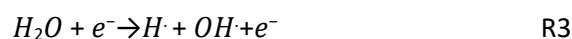
Oxidation reactions

RONS species initiate oxidation reactions such as R4. This leads to formation of $OH\cdot$ radicals because of electron dissociation. Further combination reactions produce stable products like H_2O_2 (R5). Formation of hydrogen peroxide increases oxidizing capacity of plasma and leads to production of $OH\cdot$, peroxynitrite, and HO_2 (Surowsky et al., 2015).

$$H\cdot + OH\cdot \rightarrow H_2OH\cdot + OH\cdot \rightarrow H_2O \qquad\qquad \text{R4}$$

$$OH\cdot + OH\cdot \rightarrow H_2O_2 \qquad\qquad \text{R5}$$
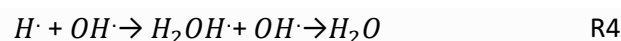
Dissociation reactions between N_2, O_2, and water lead to formation of nitrogen oxides. Nitrogen oxides further form nitrite ions as shown in R6. Reaction of nitrite ions with H_2O_2 forms nitrate ions and peroxynitrites (R7 and R8) (Thirumdas et al; 2018). Production of RNS (nitrate, nitrite ions, and peroxynitrites) contribute to lowering the pH of water. Peroxynitrites act as oxidizing agent and cause oxidizing stress on the living tissues (Surowsky et al., 2015). On interaction with the cell membrane they can cause lipid peroxidation and can diffuse into the cell causing cell death. They are also capable of causing mutagenic and cytotoxic effects on microbial cells (Thirumdas et al., 2018). Above pH 6.8, peroxynitrites have direct oxidizing effect. Below this pH peroxynitrites are highly unstable and decompose into highly reactive NO_2 (Surowsky et al., 2015).

$$O_2^- + NO^. \rightarrow (O=N-OO^{-.}) \qquad \text{R6}$$

$$NO_2^- + H_2O_2 \rightarrow (O=N-OO^{-.}) + H_2O \qquad \text{R7}$$

$$OH^. + NO_2 \rightarrow (O=N-OO^{-.}) + H^+ \qquad \text{R8}$$
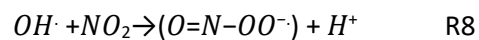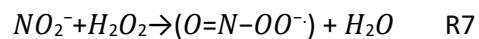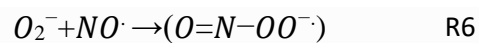
Reduction reactions

Compounds with high electron affinity are easily degraded due to presence of hydrogen radical (H$^.$) and superoxide radical ($O_2^{-.}$). These radicals initiate reduction reactions such as hydrogen addition and abstraction (Surowsky et al., 2015).

Photochemical reactions

In 190 nm - 230 nm range, several dissociation reactions occur. These are caused by vacuum UV photons. Such reactions do not take place under atmospheric conditions (Surowsky et al., 2015). These reactions are:

$$H_2O + h\nu \rightarrow H^. + OH^. \qquad \text{R9}$$

$$O_2 + h\nu \rightarrow O^. + O \qquad \text{R10}$$
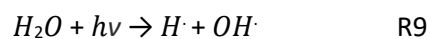
1.3.4 Plasma and its use in food production, processing, and safety
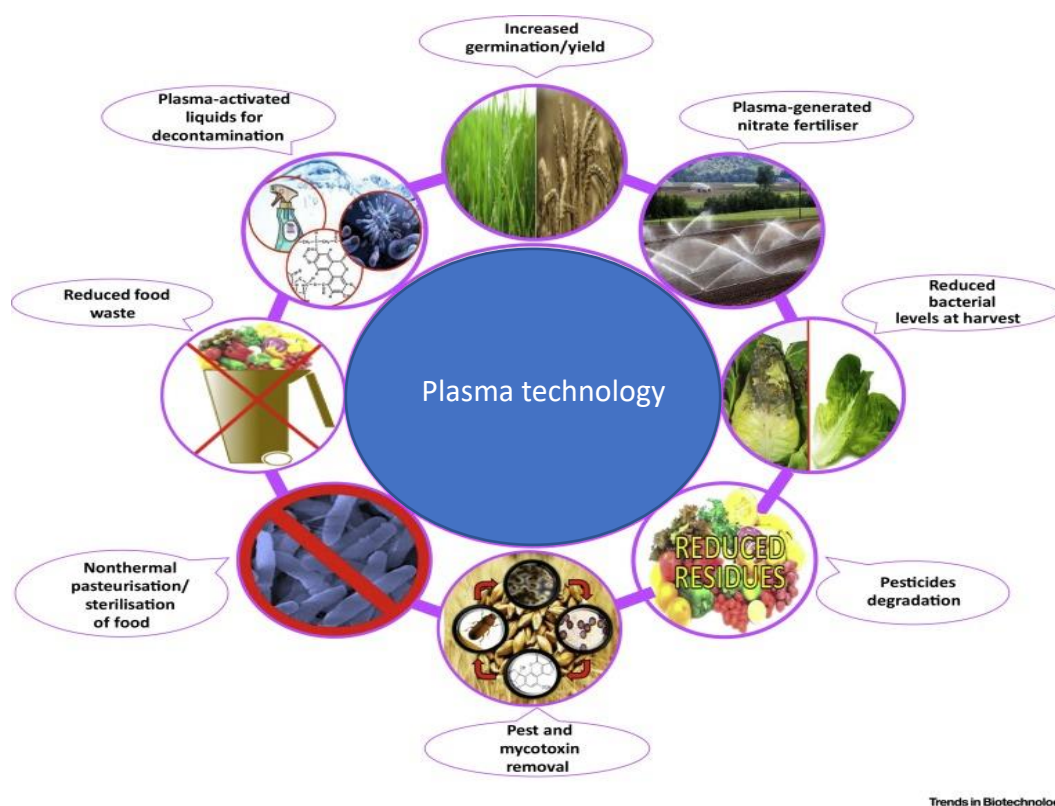
Figure 8: Depiction of applications of plasma in different stages of food production (Modified

from Bourke et al., 2018).

Plasma technology has wide applications in food production, processing, and safety (

Figure *8*). Many researchers have studied application of different types of plasma in

decontamination of seeds such as sprouts (Butscher et al., 2016), chickpeas (Mitra et al.,

2014),and rapeseed (Puligundla et al., 2017) and in improving seed germination properties in

corn (Sidik et al., 2018), rapeseed (Ling et al., 2015), artichoke (Hosseini et al., 2018), wheat

(Jiafeng et al., 2014), and peanut (Ling et al., 2016). Researchers found that plasma treatment

improved seed germination properties such as number of germinating seeds, seedling growth and aided in seed decontamination.

Kim et al. (2015) reported improved microbial quality with slight change in physicochemical quality of milk treated with dielectric barrier plasma (DBD) for 10 min. The milk sample was placed in a glass dish and was directly exposed to DBD plasma. No viable cells were found after the treatment. Milk sample inoculated with *Escherichia coli*, *Listeria monocytogenes*, and *Salmonella Typhimurium* showed 2.4 log CFU/ml reduction after 10 min treatment. Plasma treatment led to increase in L* and b* and decrease in a* Hunter color values of the treated milk.

Ziuzina et al. (2016) used open-air DBD plasma to decontaminate in-package cherry tomatoes inoculated *with E coli*. This study was done to demonstrate the potential use of cold plasma in decontaminating produce surface on industrial scale. 5.7 log CFU/sample reduction of *E. coli* population was achieved using 150 s treatment time with no significant difference in L*, a*, and b* Hunter color values of treated tomato sample.

Plasma has also been used to degrade pesticide residues on produce surface. Misra et al. (2014) studied the effect of (DBD) plasma on degradation of pesticide residues on strawberries immersed in pesticide solution. They reported significant decrease in pesticide residues detected using optical emission spectroscopy.

Plasma oxidation is an innovative technique to treat effluents from dairy, meat and poultry industry. The effluent usually contains high organic load and is traditionally treated using physiochemical or biological methods (Sarangapani et al., 2018). Doubla et al. (2007) reported significant reduction in biochemical oxygen demand of brewery effluents using gliding arc

plasma (100 W). They attributed this reduction to presence of NO· and OH· radicals. Anderson et al. (2013) and Kinoshita et al. (1997) have reported potential use of plasma in odor removal from pig house ventilation and tobacco factory emission, respectively.

Many researchers have explored the use of plasma in preventing and inactivating mycotoxin producing spores (Sarangapani et al., 2018; Dasan et al., 2016; Ouf et al., 2015). Devi et al. (2017) studied the effect of RF cold plasma with air as a gas (60 W, 30 min) on peanuts inoculated with *Aspergillus flavus* and *Aspergillus parasiticus.* They reported 99.9% and 99.5% reduction in spores in *Aspergillus flavus* and *Aspergillus parasiticus*, respectively.

1.3.4.1 Plasma and its effect on water quality

Use of plasma bubbling in hydroponic culture has shown up to 6 log CFU/ml reduction of *E. Coli*, *E. faecalis*, and *S. aureus* (Kawano et al., 2016). Algal growth in the water can lead to drastic effects such as change in pH of water, depletion of dissolved oxygen and nutrients, and formation of odor causing compounds resulting in toxic conditions. Kim et al. (2019) studied the effect of cold plasma on micro algae biomass by using chlorophyll-a, suspended solids, and turbidity as indicators of algal biomass. Their study demonstrated over 90.5 % algae removal. They further verified the removal mechanism by using electron microscope to observe the algae surface and found that at first, plasma treatment caused deformation in the surface followed by cell breakage, and shrinkage. Gnapowski et al. (2012) studied the effect of atmospheric pulse power discharge plasma on algae present in water and reported significant decrease in the algae population. Mizukoshi et al. (2018) used plasma generated by custom made bipolar high voltage pulsed power supply to treat *Chaetoceros gracilis* and *Chaetoceros calcitrans* and reported up to

100 % algae reduction, quantified after counting decolored cells with the help of neutral red dye, hemocytometer, and optical microscope.

1.3.4.2 Plasma and its effect on seed properties and plant growth

Traditional approach to improve crop yield involves increased use of fertilizer, irrigation, and seed treatments (Penado et al., 2017; Sivachandiran et al., 2017). Seeds are often treated by physical means such as soaking in hot water, ultraviolet treatment and/or chemical means such as use of insecticides, fungicides, and hormones. Such treatments are time consuming and are not uniform. This traditional approach to seed treatment is not environmentally friendly and involves use of lot of money (Hosseini et al., 2018). Plasma treatment is uniform, fast, and non-destructive method which can improve seed properties and lead to desirable change in plant growth characteristics without any adverse effect on the environment. Apart from aid in plant growth, use of plasma has an added benefit of microbial inactivation (Hosseini et al., 2018; Sivachandiran et al., 2017). Extensive research has been done on the effect of plasma on wheat seeds as it is one of the most widely consumed grain in the world. Kordas et al. (2015); Rahman et al. (2018); Ziuzina et al. (2018); Guo et al. (2018); Li et al. (2017); Roy et al. (2018); Dobrin et al. (2015); Meng et al. (2017); Zhang et al. (2018); Gidea et al. (2017) studied effect of cold plasma on wheat seed germination and seedling growth and found increased germination and growth on applying different plasma treatments.

Table 13: Effect of plasma on seed properties and seedling growth

Discharge description	Commodity used	Exposure time	Seed/seedling growing conditions	Results	Reference
Nitrogen RF cold plasma 10 W	Artichoke seeds	Seeds treated for 15 min with nitrogen plasma	Seeds incubated at 22 °C, 12 h/12 h photoperiod	Parameters measured after 7 days incubation showed 48 % increase in shoot dry weight, 11.7 % increase in germination rate	Hosseini et al. (2018)
Helium RF cold plasma 80 W	Wheat seeds	Seeds treated for 15 s with helium cold plasma	Seeds incubated at 25 °C for 7days, seeds sown in medium level fertilized field,	6 % increase in seed germination rate, 7 % increase in fresh weight of plants, 21.8 % increase in plant	Jiafeng et al. (2014)

			analyzed after 4 months	height, 13 % increase in leaf area, 5.8 % increase in total yield	
Helium RF cold plasma 120 W	Peanut seeds	Seeds were treated for 15 s	Seeds incubated at 25 °C for 7 days, seeds sown in fertilized red soil and plant characteristics analyzed at mature stage	21 % increase seed germination rate, 6 % increase in plant height, 10 % increase in stem diameter, 8 % increase in number of branches, 13 % increase in number of pods, 10 % increase in peanut yield	Ling et al. (2016)
Helium RF cold plasma 80 W	Soybean seeds	Seeds treated for 15 s with	Seeds incubated at 25 °C for 7 days	14.3 % increase in the seed germination	Ling et al. (2014)

		helium cold plasma		rate, 13.7 % and 29.9 % increase in seedling shoot length and dry weight, respectively	
Dielectric barrier discharge 6120 V	Tomato seed	Seeds treated for 6 s	Plants grown in soil	15 % increase in plant height, 18.75 % increase in fruit weight, 26.56 % increase in fruit yield	Zhou et al. (2011 b)
Capacitive coupled plasma	Rapeseed	Drought stressed seeds treated for 15 s	Seeds incubated at 25°C for 7 days	6.25 % increase in germination rate, 42.71 % and 16.6 % increase in seedling height and dry weight, respectively	Ling et al. (2015)

Pressure plasma jet	Rice	Seeds treated for 3 s	Seeds germinated at ambient conditions for 72 h	46 % increase in rice germ length	Penado et al. (2017)
Plasma plume system with helium gas	Corn	Seeds treated for 3 min	Seeds planted in soil, soil T (20 ± 5) °C, 82 % relative humidity	Seed germination time was reduced by 3 days, 3.94 % increase in plant height	Sidik et al. (2018)
Dielectric barrier discharge air plasma	Mung beans	Seeds treated for 10 min	Seeds incubated at 25 °C for 4 days	100 % increase in sprout length	Zhou et al. (2016)

From Table 13, it can be seen that lot of research has been done on plasma and seed properties and germination. The studies mentioned in above table involve use of different plasma generation technologies, voltages, treatment times, and type of seeds. In all of these studies researchers have used plasma treatment only at the beginning of the experiment to treat seeds such as wheat, rice, corn etc. After the initial treatment the seeds were germinated

and/or grown further using water in varying temperature, relative humidity, and light conditions. The seedlings were grown for few days to few weeks.

Sera et al. (2010) studied effect of microwave plasma (500 W) on different seed types. They found different response in terms of surface erosion, germination, and shoot length for wheat and oat seeds with wheat seeds being more sensitive to the treatment. In the same study treatment times from 3 min - 40 min was used. They observed negative effect on the seed properties such as number of germination seeds for longer treatment times. Root and shoot length for wheat seedlings decreased with increase in treatment time. Treatment times of 10 min - 40 min resulted in much lighter wheat seeds as compared to 3 min treatment time and control. Jiafeng et al. (2014) used RF plasma of different power (60, 80, and 100) W to treat wheat seeds and found that 80 W power gave the best results in terms of seed germination rate, plant height, fresh weight, and total yield. Sidik et al. (2018) used plasma plume system with helium gas to treat corn seeds for (3, 5, and 10) min. They found that corn seedling shoot length for 3 min treated seeds was significantly more than untreated seeds but for (5 and 10) min treatment there was considerable (39.3% and 16.8%, respectively) reduction in shoot length as compared to untreated seeds. Thus, from the above research it can be seen that effect of plasma on seed properties and responses depends on type of seed treated, type of plasma, treatment time, and power used.

Very few studies have used plasma activated water with or without seed treatment for further plant growth. Park et al. (2013) used three types of plasmas to study their effect on plant growth and nutrition. They used underwater spark discharge (2 kV, 600 mA, 5 Hz, 2 min), transferred arc discharge (1.6 kV and 300 mA, 2 min), and gliding arc plasmatron (800 V, 300 mA, 2 min) to water watermelon seeds, zinnia seeds, alfalfa seeds, pole beans seeds. For all the

treatments, seeds were planted in starter pots containing soil and were grown for 3 weeks.

Plasma activated water was fed to all pots on Monday and Wednesday (30 ml) and Friday (40

ml). These plants were harvested and their stem and root lengths, weight were measured.

Spring water treated using plasmatron had most significant effect on most the tested

parameters for watermelon, zinnia, alfalfa, and pole beans. Based on these results, gliding arc

plasmatron was further used to treat radishes, tomatoes, and banana peppers. In this part of

the experiment effect of plasma activated water was compared with fertilizer, and plasma +

fertilizer. For radish, plants treated with only plasma water showed more root and stem length

and weight as compared to fertilizer, and plasma + fertilizer. For pepper and tomato, plants

treated with plasma + fertilizer showed more root and stem length and weight as compared to

only plasma water and only fertilizer. Park et al. (2013) concluded that plasma treatment could

influence plant growth but its effect is not the same for all types of plants.

Sivachandiran et al. (2017) studied the effect of seed treatment by plasma and use of

plasma activated water on growth of radish, tomato, and sweet pepper. Dielectric barrier

plasma (40 kV, 1 kHz) was used to treat seeds for 10 min (P10) and generate plasma activated

water for 15 and 30 min (PAW15 and PAW30). After the initial seed treatment, all the seeds

(treated and untreated) were kept in a dark room (22 °C, 75 % relative humidity) and watered

using tap water (TW), PAW15 and PAW30. For plant growth, seedlings were further planted in

pots containing soil substrate (coco fiber, TRUFFAUT) and watered using tap water, PAW15 and

PAW30. The pots were kept near a window. For radish, the seed germination rate was 40 %, 60

%, and 100 % for TW, PAW15, and PAW30, respectively, for untreated seeds. For plasma treated

seeds (P10), the average seedling growth and germination rate was significantly higher (P10-

PAW30, P10-PAW-15, P10-TW) as compared to untreated seeds (PAW-30, PAW-15, TW) (see

Figure 9).
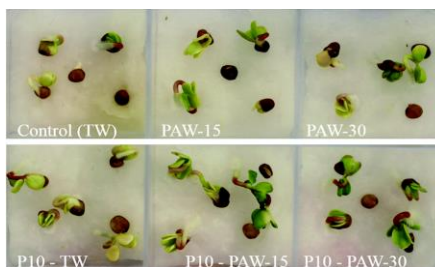
Figure 9: Radish seed germination, top row- untreated seeds (TW, PAW-15, PAW-30), bottom row- treated seeds (P10-TW, P10-PAW15, P10-PAW30) (Source: Sivachandiran et al., 2017).

Radish plants were harvested after 9 days. P10-PAW15 showed highest growth in terms of stem length among all the different treatments. And P10-PAW30 showed the least. For tomato, plants were harvested after 20 days and showed different growth pattern as compared to radish. TW and P10-TW showed highest growth as compared to rest of the treatments. To evaluate long term effects of plasma treatment, tomato and pepper plants (TW, PAW30, P10-PAW30) were grown for 60 days. For the first 9 days they were watered with PAW30 and then with TW.
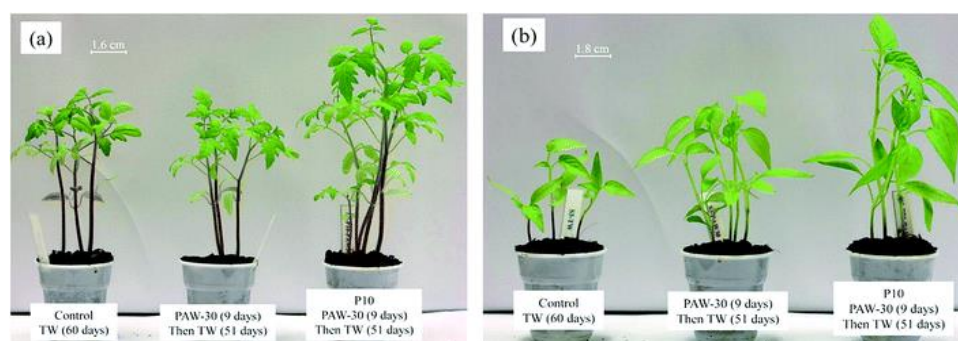
Figure 10: Plants grown using TW, PAW30, and P10-PAW30 on 60th day (a) Tomato plants (b) Pepper plants (Soure: Sivachandiran et al., 2017).

P10-PAW30 showed significantly higher growth in tomato and pepper as compared to TW, PAW-30 (Figure 10). For pepper, TW showed lowest growth thus indicating clear beneficial

effect of PAW-30 and P10-PAW30. This was not the case for tomato as there was no significant difference in terms of growth between TW and PAW30 (Figure 10). From this study it can be concluded that there is a need to optimize plasma treatment time for each type of seed and also effect of plasma water is not similar on all types of plants.

Bafoil et al. (2018) studied effect of low temperature helium plasma (10 kV, 9.7 kHz, 15 min) on *Arabidopsis thaliana* plant growth. These plants were grown in growth chamber (24 °C/20 °C, 75 % relative humidity, 16 h photoperiod) and were harvested after 42 days. Plants were watered every 2 days using tap (TW), distilled water (DW), plasma activated tap water (PAWT), and plasma activated distilled water (PAWD). Parameters such as leaf area, number of flowering plants, rosette diameter, and number of leaves were evaluated. Only PAWT showed significant increase in all the parameters (Figure 11). Bafoil et al. (2018) attributed this effect to presence of long-lived reactive nitrogen species in the PAWT. Between TW, DW there was no significance difference found. The interesting result from this study was PAWD showed similar results as that of TW, DW.

Figure 11: *Arabidopsis thaliana* growth on day 28, (A) watered using TW, (B) watered using PAWT (Source: Bafoil et al., 2018).

Thus, from the all the above-mentioned research it can be concluded that plasma treatment has great potential to replace traditional chemical methods in improving seed and

plant biological responses such as seed germination, germ length, plant height, leaf area, and total yield.

1.3.4.2.1 Mechanism of how plasma affects seed properties and plant growth

Various plasma technologies are used to generate H_2O_2, NO_x and HNO_x species (Zhou et al., 2016). These can be dissolved in water and then applied to seeds or plants, or seeds can be directly subjected to plasma which is the case for all the studies mentioned in the Table 13. It is known that reactive oxygen and nitrogen species affect cell functions such as cell differentiation, proliferation, and apoptosis. They also affect release of growth. These species can also act as signaling molecules factors (Dobrynin et al., 2009; Zhou et al., 2016).

There are many ways in which plasma can affect seed surface properties and germination. The exact mechanism by which plasma helps to change seed properties and germination is not clearly understood (Sera et al., 2010). Many variables such as type of plasma used, gas and its pressure, power values, treatment time have direct effect on this (Dobrin et al., 2015). Also seeds type plays very important role in how plasma affects seed properties (Sera et al., 2010). Researchers have reported increase in number of germinating seeds, reduced germination time, and increase in seedling growth by using cold plasma treatment (Sera et al., 2010; Ling et al., 2014; Jiafeng et al., 2014).

Generation of reactive nitrogen species leads to drop in the pH of water, this acidified water changes seed surface properties and results in rougher surface and also helps to decontaminate the surface (Zhou et al., 2016). Sera et al. (2010) hypothesized that seed coat acts as a partially permeable membrane and allows passage to some reactive species. Filatova et

al. (2013) used scanning electron microscope to detect surface change (Figure 12) in plasma treated seeds and they found that treated seeds had eroded surface as compared to control. The original reticulate surface texture of the control seeds was lost after plasma treatment. Similar results were obtained by Bafoil et al. (2018) (Figure 13). Plasma treatment results in more hydrophilic surface thus reducing the water contact angle. Hosseini et al. (2018) reported reduction from 112 to 0 in contact angle after 15 min RF plasma treatment for artichoke seeds. Researchers believe that this helps the seed to absorb more water (Sera et al., 2010; Zhou et al., 2011 b) and nutrients resulting in improved germination response (Zhou et al., 2016). Figure 14 shows more water wetting in plasma treated seeds as compared to control. Also, modification in the seed surface enhances the oxygen transmission (Sera et al., 2008). When applying direct plasma treatment, the reactive species generated in the plasma discharge penetrate the seed capsule and speed up nutriment decomposition inside the seed (Zhou et al., 2011 b). Plasma treatment of seeds could also result in more α amylase and protease activity leading to accumulation of soluble sugars and protein resulting in more growth of seedlings (Ling et al., 2014).
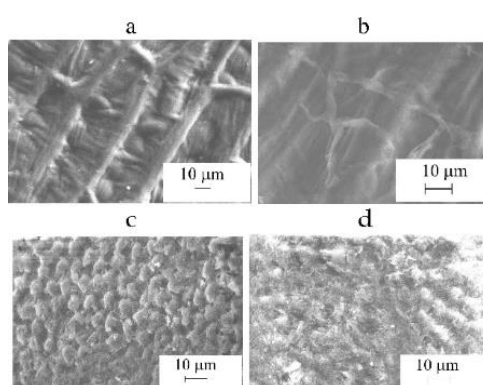
Figure 12: Scanning electron micrograph (SEM) images of seed surface of wheat (a, b) (magnification 2.00 KX) and blue lupine (c, d) (magnification 1.00 KX) for control (a, c) and plasma (b, d) (Source: Filatova et al., 2013).
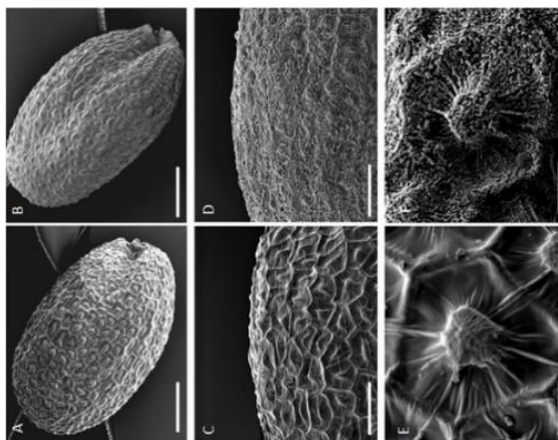
Figure 13: Scanning electron microscopy (SEM) images of *A. thaliana* seeds- (A, C, E) are the control while (B, D, F) are the air plasma treated seeds Scale bars: 100 μm A, B; 50 μm C, D; 10 μm E, F. (Source: Bafoil et al., 2018).

Figure 14: Wheat seed surface after wetting by water (a) plasma treated seeds (b) control (Source: Sera et al., 2010).

There has been extensive research conducted on the effect of plasma on seeds and how plasma affects seed properties. However, the effect of plasma on plant growth and its mechanism is not so well researched. Nitrogen is the single most important macro nutrient for plant growth. In soil, nitrogen is present in four forms- ammonium salts (NH_4^+), nitrates (NO_3^-), proteins, and products of protein decomposition (amino acids, amines, peptides etc.). Presence

of reactive nitrogen species especially NOx provides excellent growth environment (liquid nitrogen fertilizer) for plants. This can lead to increased activity of key nitrogen acquisition enzymes glutamine synthetase and nitrate reductase present in plants (Zhou et al., 2016). Brar et al. (2016) reported four-fold increase in nitrate reductase activity of plasma watered plants as compared to control. According to Sarinont et al. (2017) long lived ROS and RNS species are responsible for aid in the plant growth.

1.4 Rationale, hypothesis and objectives

Food security for future generations is one of the major problems faced by the mankind. To ensure food security, soil is one of the most valuable resource which is under lot of stress due to climate change and population explosion. This creates urgent need to practice alternative growing practices such as hydroponics. Hydroponic farming has a potential to alleviate food security problem. Global hydroponic crop value is estimated to be close to $ 30 billion in 2020 (Research and Market, 2015). Basil is one of the most widely cultivated herbs using hydroponics and is a high value specialty horticulture produce. It is one of the most used herbs prized for its taste and aroma in the world.

In hydroponic basil cultivation, control of root infecting pathogen once it gains entry to the system is very difficult because of genetically uniform produce, controlled temperature and humidity, ease of spread through nutrient solution, and root to root contact. This is even more likely for recirculating closed system (Stanghellini and Rasmussen, 1994). Due to abundant availability of recirculating nutrient water, lot of algae growth is seen in the feed water and on

the plant roots. This depletes the nutrients that are supposed to be used by basil plants. Also, as plants start to grow, they take up more and more nutrients. Thus, in closed hydroponic systems it is very difficult to provide proper nutrition to growing plants.

Plasma treatment of recirculating hydroponic solution can be an effective method to reduce microbial load of the solution, cause algae removal, and add RONS which can aid in the basil plant growth. Hydroponic growers can get more basil yield due to reduction in plant diseases and algae buildup. Plasma treatment has a great potential to replace traditional disinfection methods such as UV, ozone, and heat treatment which are not uniform and are not sustainable. Plasma treatment offers added benefit of more plant growth due to presence of reactive nitrogen species. With use of plasma treatment, more and more growers can opt for recirculating closed hydroponic systems instead of open systems which is a more environmentally friendly hydroponic growing method.

The overall aim of this study was to design and develop a lab-scale hydroponic system for sweet basil to investigate the effect of plasma activated nutrient solution on basil growth and quality parameters.

The specific objectives were:

- To design and develop two-lab scale hydroponic chambers to grow basil: one to grow basil using a standard nutrient solution (NS) and the other to grow basil using plasma activated nutrient solution (PANS);
- To measure and compare the growth of basil in terms of height, fresh weight, leaf index, nodes, branches between NS and PANS treatments;

- To evaluate and compare the quality of basil in terms of color, texture, yield, aroma

  compounds, and tissue nutrients between NS and PANS treatments; and

- To assess the effect of PANS on algae content between the two treatments.

2. MATERIALS AND METHODS

2.1 Materials

2.1.1 Hydroponic chambers set up

2.1.1.1 Enclosed chambers

Two identical enclosed chambers were constructed using materials (Secret Jardin Dark Street v3.0) purchased from NWGS LLC, Monroe, WA. They were used in this study to grow basil, one using NS and the other using PANS. The nutrient solution chamber (NS chamber) and plasma activated nutrient solution chamber (PANS chamber) were assembled in the pilot plant of Food Science building. These chambers were 1.2 m wide, 0.6 m deep, and 1.7 m high. After the assembly the chambers were covered with black covers so that no outside light could enter the chambers. The inside of the enclosure was made of reflective material such that maximum available light could reach the plants. A zipper was used to open and close the chambers. These chambers were fixed to table tops (Figure 15), side by side, for the ease of handling and cleaning.

Figure 15: Two assembled enclosed chambers on the table tops, left- NS chamber, right-PANS chamber.

2.1.1.2 Flood table

Active aqua flood tables (Figure 16) purchased from NWGS LLC, Monroe, WA, were used to keep the plants inside the chambers. The flood tables were (1.2 × 0.6) m with inside dimensions being (1.1 × 0.54) m.

Figure 16: Active aqua flood table.

2.1.1.3 Water tank

Water tanks (Chem-Tainer rectangular tank) each of 55 L capacity were purchased from McMaster-Carr, Robbinsville township, NJ. The tanks were 0.45 m wide, 0.3m deep, and 0.45 m high. These tanks were used to store NS and PANS. The tanks were later on spray painted on the outside with a black paint to limit exposure of NS and PANS to outside light to reduce growth of algae in the stored solutions.

2.1.1.4 Water pump

Submersible pumps (EcoPlus® Eco633, 120 V, 50 Hz, 0.45 A) were ordered from Amazon

and were used to pump the NS and PANS from NS and PANS chamber tanks, respectively, to the

flood tables kept on the table tops. Inlet and outlet pipes from the pumps were attached to the

flood tables. Pumps were operated using automatic timers which switched the pumps on every

3 h, 8 times a day for 8 min at a time.

2.1.1.5 Lights

White fluorescent lights (1.2 × 0.6) m (Sun Blaze T5 HO 4 ft 120 V) each of 8 lamps

purchased from NWGS LLC, Monroe, WA, were used to keep the photoperiod of 16 h inside NS

and PANS chambers. The lights were attached to a timer which aided to keep the same

photoperiod throughout the harvest period. The lights were hanged inside the chambers using

ropes such that they could be taken up and down as plants grow to obtain more or less the

same light intensity on top of the plants throughout 21 days.

2.1.1.6 Fan

Vent blower fans (VIVOSUN 4-inch 203 CFM, 2500 RPM) were ordered from Amazon

and were used to maintain good air circulation inside the chambers. Also, the lights inside the

enclosed chamber tend to increase the temperature inside the chambers. Thus, the fans were

kept on and at high speed throughout the harvest period for air circulation.

2.1.1.7 CO_2 sensor

K33 ELG CO_2 sensors purchased from CO2Meter.com, Ormond Beach, FL, were used to measure carbon dioxide levels inside the chambers. The range of the sensor was 0 ppm - 10,000 ppm ± 30 ppm. The sensor was set to measure the CO_2 level after every 1 h throughout the harvest period. The data from the sensor was downloaded by connecting the sensor to a PC via USB.

2.1.1.8 Temperature and relative humidity sensor

Sensiron SHT31 sensors purchased from Digi-Key® Electronics, Thief River Falls, MN were used to measure and record the temperature and relative humidity inside the chambers. The range of the sensor was -40 °C to 125 °C ± 0.2 °C and 0 % to 100 % ± 2 % for temperature and relative humidity, respectively, with 8 s response time. The sensor was set to measure the temperature and relative humidity after every 1 h throughout the harvest period. The data from the sensor was downloaded by connecting the sensor to an Android smartphone via Bluetooth.

2.1.1.9 Temperature sensor

Onset® HOBO® 4 channel thermocouple data logger purchased from Onset®, Bourne, MA was used to measure the temperature of the NS and PANS inside NS and PANS chamber tanks, respectively. The temperature was measured by attaching two K-type thermocouple

probes (1 for each tank) to the sensor. The range of the sensor was -20 °C to 70 °C ± 0.2 °C. The

sensor was set to measure the temperature every 1 h throughout the harvest period. The data

from the sensor was downloaded by connecting the sensor to a PC via USB.

2.1.1.10 NS and PANS chambers assembly

Two identical enclosed chambers were assembled in the pilot plant of Food Science

building (Figure 17). These were kept on table tops for ease of handling and cleaning. Two holes

were drilled in the table tops to allow inlet and outlet pipes for NS and PANS. Flood tables were

placed inside the assembled chambers and the inlet and outlet pipes from the water pump were

attached to the flood tables. Water tanks were kept below the table tops, on the ground. A

water pump was placed on the bottom of each of the tanks and was attached to a timer. The

lights panels were hanged inside the chambers with ropes so that the height of the panels could

be adjusted. Each light panel had 8 white tubes. The lights were also attached to a timer.

Exhaust fans were located on top of the chambers to allow enough air circulation inside. The

CO_2 sensor, one in each chamber, was glued to the top inside of the chambers to measure the

CO_2 concentration inside the chambers. The temperature and relative humidity sensor, one in

each chamber, was placed on the bottom inside the chamber to measure the temperature and

relative humidity inside the chambers. The temperature sensor for recording temperature of NS

and PANS in the tanks was kept outside of the chambers and on the table top. Two K-type

thermocouple probes one for each chamber were attached to the temperature sensor which

aided to measure temperature of NS and PANS inside the chamber tanks.
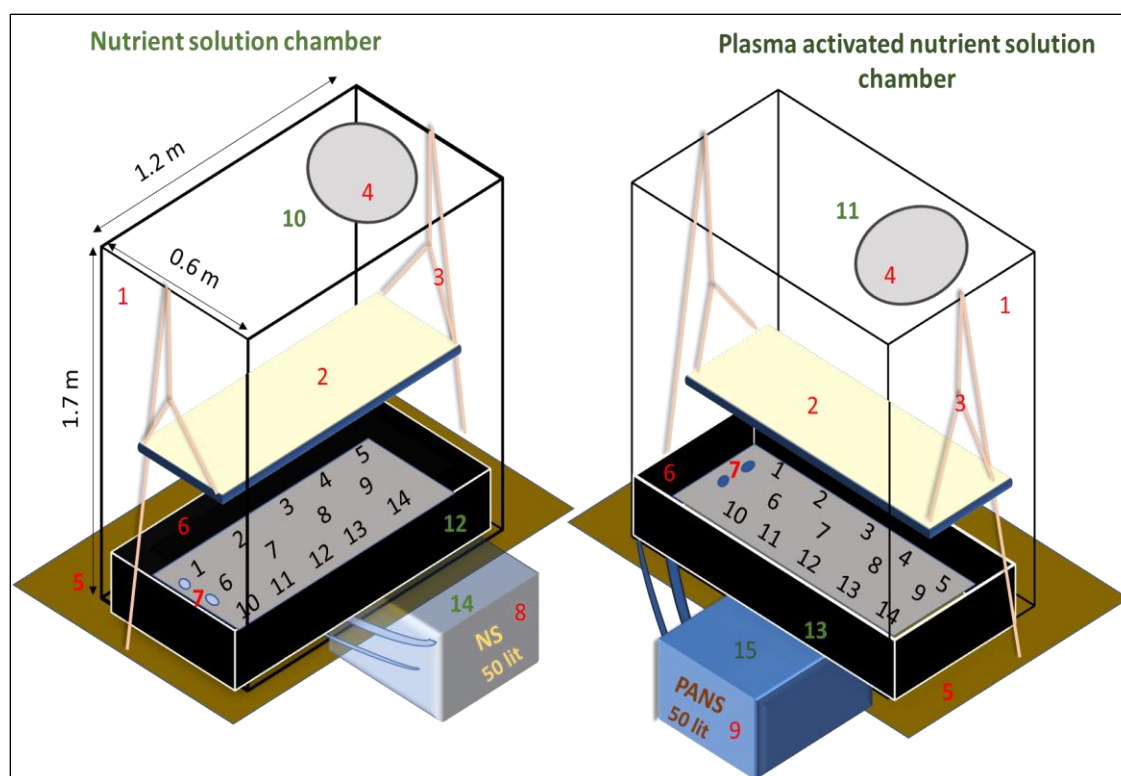
Figure 17: Assembled chambers, left: NS chamber, right: PANS chamber.

1- Enclosed chambers (1.2 × 0.6 × 1.7) m

2- White fluorescent lights (1.2 × 0.6) m

3- Adjustable ropes used to hang the lights

4- Fans

5- Table tops used to keep the chambers

6- Flood table showing placements of 1-14 basil plants

7- Inlet and outlet pipes from the tanks connected to the flood tables

8- NS chamber tank with 50 L NS

9- PANS chamber tank with 50 L PANS

10, 11- CO_2 sensors

12, 13- Temperature and relative humidity sensors

14, 15- Temperature sensors

2.1.2 Seedling and plant growth

2.1.2.1 Basil seeds

The commercial variety "Rutgers Devotion DMR" basil seeds were purchased from Van Drunen Farms® VDF Specialty Seeds®, Momence, IL. This basil cultivar was developed at Rutgers University by James E. Simon, Robert Pyne and C. Andrew Wyenandt (U.S. Utility Patent: 10159212). This is a Genovese type basil with large and cupped leaves. In moderately rich and moist soil condition, it takes 5-10 days to germinate and 70-80 days to mature.

2.1.2.2 Rockwool slab

Grodan A-OK rockwool stonewool hydroponic grow media starter cubes ordered from Amazon were used to grow basil seedlings for NS and PANS chambers. Rockwool slab was used as it retains water, provides good aeration because of the fibrous, porous structure which aids in seed sprouting and healthy root growth. A single slab consisted of 50 small rockwool blocks (Figure 18 a and Figure 18 b). Each block had a small hole on top approximately 0.5 cm deep where Rutgers Devotion DMR basil seeds were placed for sprouting. The weight of the single slab was approximately 70 g which increased up to 800 g after soaking it in water for a minute. The rockwool slab was placed in a plastic tray which was kept inside a growth chamber to grow basil seedlings.
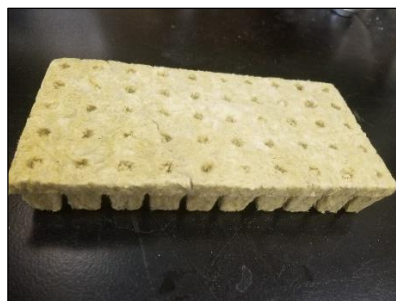
(a)   (b)

Figure 18: (a) A single rockwool block cut from the rockwool slab, (b) Rockwool slab consisting of 50 small rockwool blocks.

2.1.2.3 YaraLiva® Calcinit™

YaraLiva® Calcinit™ fertilizer was ordered from Amazon. Nitrogen is the single most important nutrient necessary for plant growth. Calcium is one of the few macronutrients required for plant growth. YaraLiva® Calcinit™ is a soluble grade calcium nitrate used to provide good nutrition for seedling and plant growth. YaraLiva® Calcinit™ (total nitrogen- 15.5 %, soluble calcium-19.0 %) was used to prepare stock A (section 2.2.2). This fertilizer was the primary source of nitrogen and calcium for the seedlings and plants. The granular form of the fertilizer aided in its fast dissolution in the water.

2.1.2.4 Jack's® Nutrients

Jack's® Nutrients 5-12-26 fertilizer formula was ordered from Amazon. It was used to provide macro (phosphate 12 %, potash 26 %, Mg 6.3 %, S 8.5 %) and micro (B 0.05 %, Cu 0.015

%, Fe 0.3 %, Mn 0.05 %, Zn 0.015 %, Mo 0.019 %) nutrients required for seedling and plant

growth. This fertilizer was used to prepare stock B (section 2.2.2).

2.1.2.5 Growth chamber

In this study, a growth chamber (Figure 19 a and Figure 19 b) located at Rutgers

greenhouse on Cook campus was used to grow basil seedlings. The growth chamber was

maintained at

25 °C, 75 % relative humidity, and 100 µmol/m^{2}s light at all times. The rockwool slab with basil

seeds was kept in a plastic tray for 14 days inside the growth chamber.

(a)   (b)

Figure 19: Growth chamber (a) From outside, (b) Inside of the chamber showing the plastic tray

where rockwool slab with basil seeds was placed.

2.1.2.6 Grodan blocks

Grodan delta 4 rockwool blocks (7.5 cm wide, 7.5 cm deep, and 6.3 cm high) purchased from Amazon were used (Figure 20 a) at the time of transferring the seedlings from growth chamber to NS and PANS chambers on 14th day. Weight of each grodan block was approximately 21 g and after soaking in water for a minute it increased to 222 g. Each block had approximately 4 cm deep hole on the top such that a single rockwool block could perfectly fit inside of it (Figure 20 b). Fourteen (14) such grodan blocks were used in each chamber.

(a)   (b)

Figure 20: (a) Grodan block, (b) Grodan block with rockwool block placed in it.

2.1.2.7 Phosphoric acid

Phosphoric acid 10 % v/v (LabChem Quality Solution, Zelienople, PA) was used to adjust the pH of NS and PANS to 5.8.

2.1.2.8 Potassium hydroxide

10 % solution of Potassium hydroxide (≥ 85 % pellets Sigma Aldrich®) were used to adjust the pH of NS and PANS to 5.8.

2.1.3 Materials used for measurement and analysis of NS and PANS solutions, and basil

plants

2.1.3.1 Sodium nitrate

Sodium nitrate (≥ 99 % purity) was obtained from Fisher Scientific™. It was used for

nitrate measurement using Spectroquant® assay explained in detail in section 2.2.8.2.1. The

reagent was diluted with distilled water to obtain concentrations: 1.7 mM, 3.2 mM, 6.4 mM, 9.7

mM. These solutions were then used to obtain the standard curve to estimate nitrate

concentration of the sample using its absorbance values at 340 nm.

2.1.3.2 Sodium nitrite

Sodium nitrite (≥ 97 % purity) was obtained from Fisher Scientific™. It was used for

nitrite measurement using Spectroquant® assay explained in detail in section 2.2.8.2.2. Sodium

nitrite was diluted with distilled water to obtain concentrations: 1.2 mM, 2.5 mM, 3.8 mM, 5.1

mM, 6.4 mM. Further a standard curve was obtained using these solutions to estimate the

nitrite concentration of samples using their absorbance values taken at 451 nm.

2.1.3.3 Hydrogen peroxide solution

Hydrogen peroxide solution (30 wt. % in H_2O, ACS reagent) was obtained from Fisher Scientific™.

It was diluted with distilled water to obtain concentrations: 0.03 mM, 0.06 mM, 0.1 mM, 0.3

mM, 0.45 mM, 0.6 mM. These solutions were used to obtain a standard curve to estimate the

concentration of hydrogen peroxide in the sample using their absorbance values at 410 nm. The

absorbance values were obtained using Spectroquant® assay explained in detail in section

2.2.8.2.3.

2.1.3.4 Assay kits

2.1.3.4.1 Nitrate assay

Spectroquant® nitrate cell test purchased from MilliporeSigma, Burligton, MA, was used

to measure the nitrates present in NS and PANS. The assay procedure explained in section

2.2.8.2.1 was followed and then the sample was immediately subjected to photometric

measurement as increased reaction time of the assay interferes with the photometric reading.

The assay kit was stored at room temperature. Proper care was taken to avoid any direct

contact with the assay material.

2.1.3.4.2 Nitrite assay

Spectroquant® nitrite cell test purchased from MilliporeSigma, Burligton, MA, was used

to quantify the nitrites present in NS and PANS. The photometric analysis of the sample was

done immediately after completion of the assay reaction time (explained in section 2.2.8.2.2) to

avoid any false results. The assay kit was stored at room temperature.

2.1.3.4.3 Hydrogen peroxide assay

Spectroquant® H_2O_2 cell test purchased from MilliporeSigma, Burligton, MA, was used to measure the hydrogen peroxide present in NS and PANS. Just like the nitrate and nitrite assays the photometric measurement was done immediately after the reaction time (explained in section 2.2.8.2.3). The assay kit was stored at room temperature.

2.1.3.5 Test strips

2.1.3.5.1 Nitrate test strips

Insta-Test® Analytic nitrate strips (0 ppm - 200 ppm ± 10 ppm) purchased from Amazon were also used to quantify the nitrate content of NS and PANS. The test strips were used for their ease of measurement and handling. Nitrate reading was obtained by matching the color of the tested test strip to a color chart (Figure 21). These test strips were stored at room temperature.

Figure 21: A tested nitrate test trip compared against a color chart.

2.1.3.5.2 Nitrite test strips

MQuant® nitrite test strips (0 ppm - 80 ppm ± 2 ppm) purchased from Amazon were used as an easy and quick method to quantify nitrite content of NS and PANS. The final reading was obtained by matching the color of the tested strip to a color chart. These strips were stored at approximately 4 °C.

2.1.3.5.3 Hydrogen peroxide test strips

Bartovation test strips (0 ppm - 100 ppm ± 1 ppm) purchased from Amazon were used to quantify the hydrogen peroxide content in NS and PANS. It was quantified by matching the color of the tested strip to a color chart. These strips were stored at room temperature.

2.1.3.6 Electrical Conductivity (EC) meter

Hand held EC meter (HEALTH METRIC®) (Figure 22) with titanium alloy probe suitable for cold and warm liquids purchased from Amazon was used to determine the EC of NS and PANS. The range of the meter was (0-9999) µS/cm ± 2 %. The meter was factory calibrated.

Figure 22: Handheld HEALTH METRIC® EC meter.

2.1.3.7 Sedgewick cell

In this study, a Sedgewick cell (Electron Microscopy Sciences) purchased from Amazon was used to enumerate algae cells present in NS and PANS. It was a (50 × 20) mm four-sided plastic chamber, 1 mm deep (Figure 23). The total volume of the chamber was 1000 mm^3. The cell had a grid on the bottom which had lines 1 mm apart. Each grid square corresponds to volume of 1 mm^3. A glass cover slip was used to completely cover the top of the Sedgewick cell. The algae concentration was measured by placing the cell under a microscope and counting the algae cells in at least 50 grid squares to obtain an average value.
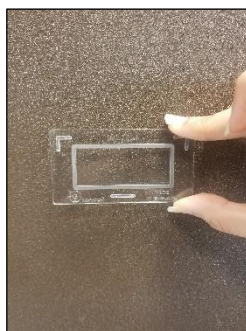
Figure 23: Sedgewick cell.

2.1.3.8 Lugol's solution

In this study Lugol's solution containing 4 % potassium iodide and 2 % iodine (Carlyle™) purchased from Amazon was used to enumerate algal cells present in NS and PANS. Lugol's solution immobilizes the algal cells and stains the starch in them, making the cells dark and heavy. This causes them to sink at the bottom of the Sedgewick cell and makes it easier to count them. This solution was stored in dark and dry place.

2.1.4 Media and solutions

2.1.4.1 Total plate count agar

For microbiological testing of basil leaves, Plate Count Agar (PCA) (obtained from MG Scientific, Pleasant Prairie, WI) was used to perform total plate count test. 23.5 g of Difco™ PCA was mixed in 1 L of distilled water in a 2 L glass bottle using a magnetic stirrer for 10 min. The bottle was then placed on a heating plate for 15 min - 30 min to completely dissolve the agar by obtaining a clear solution. The clear solution was autoclaved at 121 °C for 15 min. This sterile solution was allowed to cool up to 45 °C - 50 °C. 20 ml of sterile solution was then poured on to sterile petri plates and the plates were kept overnight at room temperature. Later, these plates were stored in a refrigerator and used as needed.

2.1.4.2 Peptone water

To perform total plate count test, peptone water was used as a buffer solution. For preparation of peptone water, 1.5 g of Difco™ peptone powder was dissolved in 1 L of distilled water using magnetic stirrer for 10 minutes. It was then kept on a hot plate for 15 min to completely dissolve the powder and obtain a clear solution. This solution was used to pipette 9 ml aliquots into glass tubes. Filled glass tubes were autoclaved at 121 °C for 15 min.

2.1.5 Instruments used in the study

2.1.5.1 Gliding arc plasmatron

Gliding arc plasmatron system was used to generate PANS by direct exposure method (given in section 1.3.2). It was developed by AA Plasma LLC, Philadelphia, PA with the help of Nyheim Plasma Institute, Camden, NJ. The plasmatron system was kept in the pilot plant of Food Science Building at Rutgers University. In the plasmatron system, the gliding arc plasma discharge was generated by plasma power supply in ionized air creating radicals, ions, and reactive species. The compounds such as N_2, NO_X were formed in the plasma discharge.
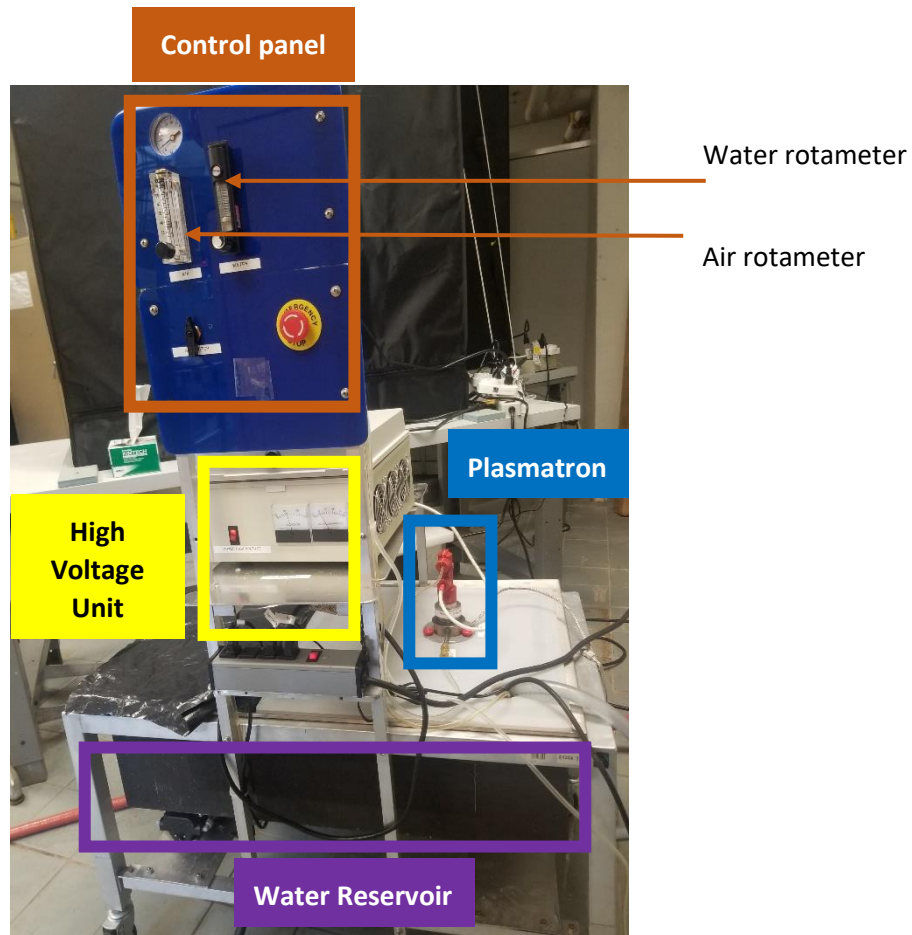
Figure 24: Gliding arc plasmatron system.

The gliding arc plasmatron system consisted of 4 main components- control panel, high voltage unit, plasmatron (torch), and water reservoir (

Figure 24). The control panel had air (SCFH) and water (GPH) rotameters to adjust the flowrate of air and water, respectively. The panel front has an air switch to switch on the air supply when generating plasma, an emergency stop button, and a pressure gage to check air pressure (psi). The high voltage unit consists of high voltage switch, general power switch on the back, ground, and analog gauge showing readings for voltage (DC V) and current (mA). The plasmatron is a torch like unit where gliding arc plasma is generated. The plasmatron is

connected to air, water, and high voltage supply. The water reservoir is made of stainless-steel

sink (70 L) and has a water submerged pump on the bottom to transfer the generated PANS to

PANS chamber tank.

2.1.5.2 Texture analyzer

A Brookfield CT-3 texture analyzer was used to perform the puncture test of basil leaves

(Fig. 25). A 2 mm diameter flat base cylindrical probe was used for the test. The base plate was

modified using two circular concentric plastic plates (seen in Figure 25) to hold the basil leaf in

place and minimize its slacking during the test. The value for peak force was obtained from the

force vs distance graph of puncture test. Leaf toughness was estimated after calculating area

under the force vs distance graph. The value of Young's modulus was calculated using the force

vs distance graph and by using Equation 3.

Figure 25: Brookfield CT-3 texture analyzer with a 2 mm diameter probe, modified base plate,

and basil leaf held between the circular plastic plates.

2.1.5.3 Colorimeter

A hand held portable colorimeter (Figure 26) with LCD display (Konica Minolta CR-410) was used to determine the color of basil leaves. D65 standard values (Y = 94.7, x = 0.3156, and y = 0.33199) were used to calibrate the colorimeter. The color was measured in terms of L*, a*, and b* values (CIE lab scale). L* represents lightness/darkness of the sample, negative a* and positive a* represent greenness and redness, respectively, negative b* and positive b* represent blueness and yellowness of the sample, respectively.

Figure 26: Konica Minolta CR-410 colorimeter.

2.1.5.4 Spectrophotometer

BioTek™ Epoch™ microplate spectrophotometer with a 96 well microplate (Figure 27) was used to quantify nitrates, nitrites, and hydrogen peroxide present in NS and PANS. This meter was equipped with Gen5™ Software interface and offered filter free, wide wavelength range measurements. The spectrophotometric measurements were done immediately after the initial assay reaction of the sample.

Figure 27: BioTek™ Epoch™ spectrophotometer with a 96 well microplate.

2.1.5.5 Light meter

A hand held portable light meter (Figure 28) with LCD display and separate quantum sensor (Apogee MQ-200) was used to measure the light inside the NS and PANS chambers. The light was measured in terms of photosynthetic photon flux density (PPFD) ($\mu mol/m^2s$). This sensor was ideal for measuring PPFD over plant canopies in outdoor and greenhouse environment.

Figure 28: Handheld portable Apogee MQ-200 light meter with a separate quantum sensor.

2.1.5.6 Microscope

Olympus BH-2 BHSP light microscope with 4x, 10x, 20x, and 40x objectives was used to enumerate algae content of NS and PANS. In this study, 10x objective was used to observe and enumerate the algae concentration.

2.1.5.7 Vacuum oven

Yamato ADP-31 PID controlled benchtop vacuum drying oven (1 cu ft, 120 VAC, stainless steel interior) (Figure 29) was used to obtain dry weight and moisture content of harvested basil plants. The tempered glass window allowed to see inside the oven while the sample was being dried. The dried samples were later on used for aroma analysis. Use of vacuum oven allowed drying at low temperature (40 °C) for better retention of volatile aroma compounds.

Figure 29: Yamato ADP-31 vacuum oven with a tempered glass window.

2.2 Methods

2.2.2 Preparation of NS

To prepare NS, stock A and stock B were mixed in desired proportions.

Stock A

194 g of YaraLiva® Calcinit™ was added to 2 L of filtered tap water in a 2 L beaker. Using magnetic stirrer, the solution was mixed at room temperature for approximately 1 h to make sure all the Calcinit™ is dissolved to obtain a clear solution. The clear solution was stored at room temperature in a plastic container and labelled.

Stock B

129 g of Jack's Nutrients 5-12-26 was added to 2 L of filtered tap water in a 2 L beaker. Using magnetic stirrer, the solution was mixed at room temperature for 1.5 h -2 h to get a clear solution. The clear solution obtained was stored at ambient temperature in a plastic container and labelled.

Stock A and B were mixed with filtered tap water in the ratio 1:1:98, respectively, to prepare full strength nutrient solution (NS) with approximate EC and pH of (1.6 - 1.7) mS/cm and 6.8, respectively.

The NS was diluted 1:1 with filtered tap water to prepare half strength nutrient solution to feed the basil seedlings with approximate EC and pH of (0.8 - 0.85) mS/cm and 6.8, respectively.

The pH of NS and half strength NS was adjusted to 5.8 using 10 % phosphoric acid. The pH adjustment was done to make sure that nutrients that were present in the solution were available for absorption to the plants as the pH of 5.8 is most favorable for nutrient absorption for basil. While handling YaraLiva® Calcinit™, Jack's Nutrients 5-12-26, and their solutions precaution was taken to limit their contact with skin and eyes; gloves, eye protection, and lab coat were worn while handling.

2.2.3 Seedling growing

A single rockwool slab consisting of 50 small blocks was used to grow seedlings for NS and PANS chambers. This slab was submerged in tap water for a minute the day before planting the seeds and excess water was allowed to drain overnight. The slab was placed in a plastic tray with approximate dimensions of (25 w x 16 d x 10 h) cm. 3 - 4 Rutgers Devotion DMR basil seeds were then placed in each of the 50 blocks by hand. 1 L tap water (pH $\approx$ 6.8) was added to tray (Figure 30 a).

(a)   (b)

Figure 30: (a) Basil seeds placed in a presoaked rockwool block placed in a tray on day 1, (b) Tray

kept inside a growth chamber at Rutgers Greenhouse on day 1.

This tray was placed in a growth chamber at Rutgers greenhouse (Figure 30 b). The

growth chamber was maintained at 25 °C, 75 % relative humidity, and 100 µmol/m^{2}s light at all

times. The seeds started sprouting after 4 - 5 days and at this stage leftover water from the tray

was discarded and the tray was filled with 1 L of half strength nutrient solution (EC ≈ 0.8 mS/cm,

pH ≈ 5.8) as the seeds need more nutrition after sprouting (Figure 31 a).
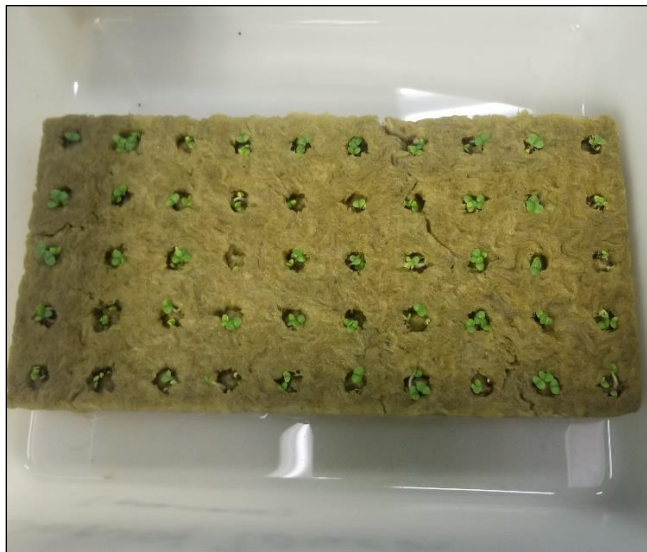
(a)

(b)

Figure 31: (a) basil seeds sprouting in rockwool slab on day 4, (b) basil seedlings growing in rockwool slab on day 10.

On day 10 (Figure 31 b) the remaining water from the tray was discarded and 1 L of fresh half strength nutrient solution (EC $\approx$ 0.8 mS/cm, pH $\approx$ 5.8) was added to the tray. This was done to make sure the nutrients were available in the correct proportion as the seedlings started to grow, they take up more nutrients. The tray was kept in the growth chamber till day 14. The height of the seedlings was approximately 4 cm - 5 cm at the end of 14 days (Figure 32). They were then transferred to NS and PANS chambers for further plant growth for 3 weeks.

Figure 32: Basil seedlings on day 14 ready for transferring to NS and PANS chambers.

2.2.4 Generation of PANS

PANS was prepared using gliding arc plasmatron system. The water reservoir of

plasmatron system was filled with 50 L of NS (pH $\approx$ 6.8) prepared as given in section 2.2.2. For

generation of PANS, the first step was turning on the air and water flow for the plasmatron

torch using the rotameters and the air switch located on the control panel unit. This was done to

make sure there were no air bubbles in the circulating water. This was followed by turning on

the general power switch and then the high voltage switch, both located on the high voltage

unit. At a particular flow rates of air and water one could hear and see the plasma being

generated (Figure 33). The air and water flow rates used in this study were 30 SCFH (0.8 m^3/h)

and 1 GPH (3.7 L/h), respectively. These flow rates were adjusted using the rotameters every 2-3

times a minute to make sure the plasma was being generated. pH of the solution in the water

reservoir was checked every 15 min. The plasmatron system was operated and flow rates were

adjusted until the pH of approximately 3.5 was reached. PANS sample was collected from the

reservoir for nitrate, nitrite, and hydrogen peroxide measurement using assays (refer 2.2.8.2) and test strips (refer 2.2.8.3). At this time the plasmatron was switched off and pH of PANS was adjusted to 5.8 using 10 % solution of potassium hydroxide.

Plasma being generated by direct exposure method

PANS in the water reservoir of plasmatron system
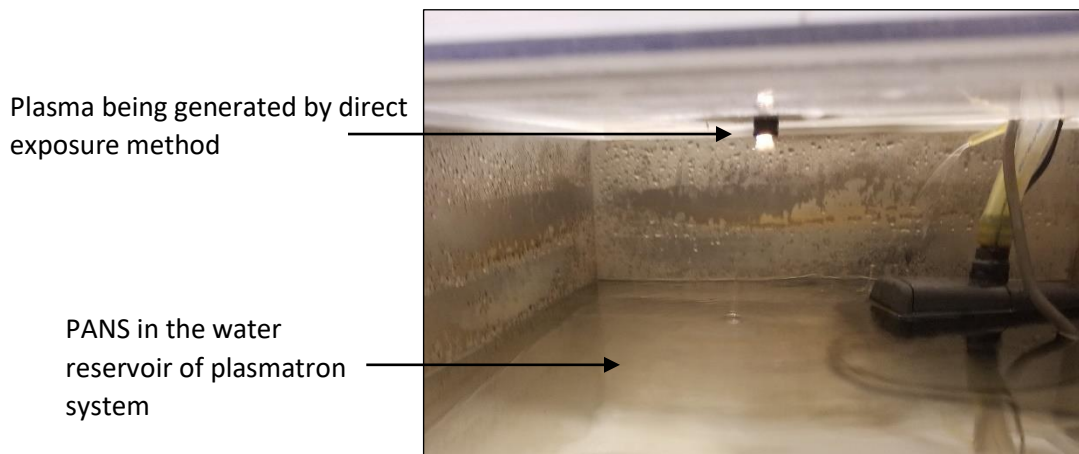
Figure 33: PANS being generated using direct exposure method by gliding arc plasmatron system.

With the help of a submerged pump (placed on the bottom of the water reservoir of plasmatron system), freshly prepared PANS was then transferred from the water reservoir to the PANS chamber tank which was placed adjacent to the plasmatron system.

2.2.5 Transfer of seedlings to NS and PANS chambers

On the 14th day the seedlings were taken out of the growth chamber. Using hands and knife each block from the rockwool slab was separated in to 50 small rockwool blocks. Extra seedlings were plucked out from each rockwool block by hand such that only 3 seedlings

remained in each of the 50 blocks. Fourteen rockwool blocks for each of NS and PANS chambers were selected randomly and each block was placed inside a grodan block (Figure 34 a). Fourteen such grodan blocks were placed inside NS and PANS chambers (Figure 34 b and Figure 35 a). The plants were randomly rotated at the start of week 2 (8th day) (Figure 35 b) and week 3 (15th day) (Figure 35 c) to reduce variability in exposure of plants to light and water.

(a)

(b)

Figure 34: (a) Seedling in a rockwool block placed inside a grodan block, (b) 14 grodan blocks placed in the flood table inside the chamber.

(a)

(b)

(c)
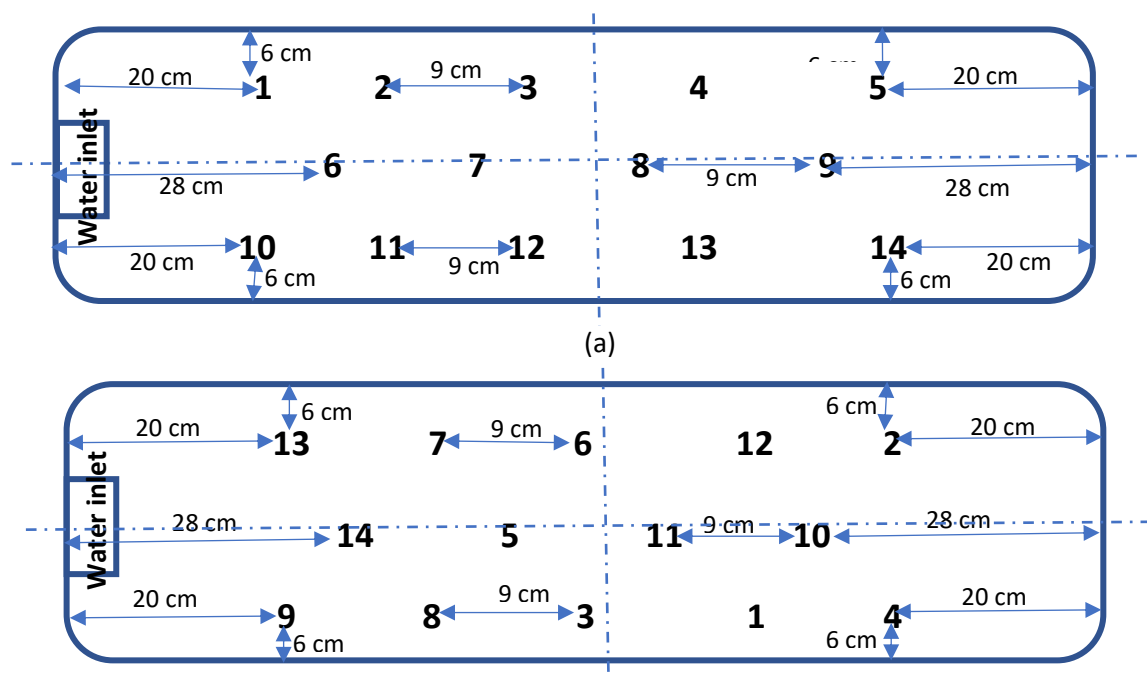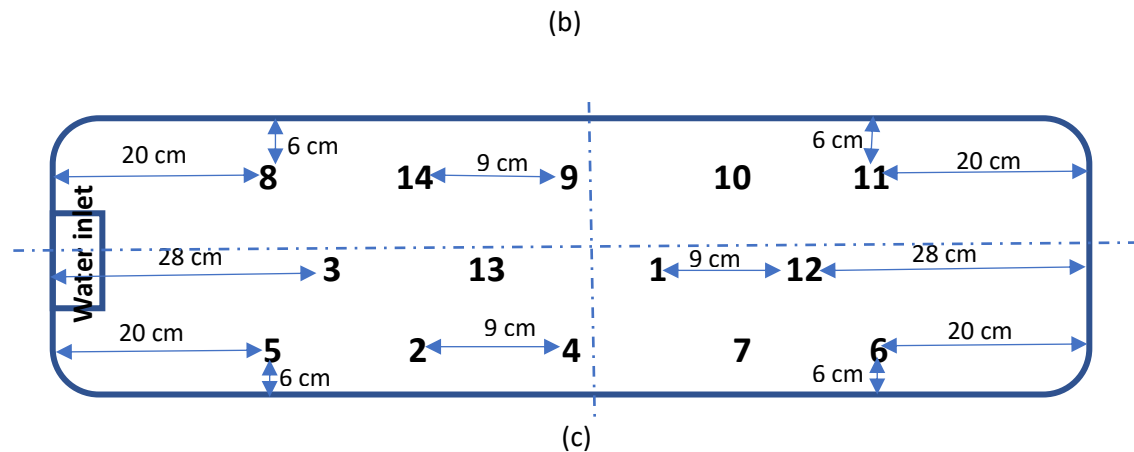
Figure 35: (a) Top view of the flood table on day 1 (week 1), showing placements of plants 1-14, (b) Top view of the flood table on day 8 (week 2), showing placements of plants 1-14, (c) Top view of the flood table on day 15 (week 3), showing placements of plants 1-14.

The light exposure period was 16 h starting from 2 PM to 6 AM, continuously. The distance between top of the plant canopy and light was kept at approximately 0.3 m to make sure the PPFD measured on top of the plants is approximately 250 µmol/m²s (measured using a handheld portable Apogee MQ-200 light meter). The NS and PANS flooding time was set at 8 min every 3 h. The CO_2 sensor, temperature and relative humidity sensor, and temperature sensor for NS and PANS were set to record the readings every 1 h. The NS chamber tank and PANS chamber tank were filled with 50 L of freshly prepared NS (as given in section 2.2.2) and PANS (as given in section 2.2.4), respectively.

2.2.6 Plant growth in NS and PANS chambers

The distance between plants and light was adjusted every week using the ropes that were used to hang the light panels inside the chambers. The temperature, relative humidity, and CO_2 inside both the chambers were not controlled but monitored and recorded every 1 h for 21 days. On day 1, 50 L of freshly prepared NS and PANS were stored inside NS and PANS tanks, respectively, which was enough to flood the flood tables for 21 days.

To see the effect of PANS on plant growth, two treatment variations were done during plant growth period. In the first treatment variation (section 2.2.6.1), PANS and NS was prepared on day 1 and the same solutions were used to water the plants throughout their harvest period of 21 days (single plasma treatment). In the second treatment variation (section 2.2.6.2), PANS and NS were prepared fresh on days 1, 8, and 15 (multiple plasma treatments) as explained in the next section. For both the treatments, the pump was set to flood the flood tables every 3 h for 8 min.

2.2.6.1 Treatment 1

For single plasma treatment, the NS was treated using gliding arc plasmatron on day 1 to prepare PANS (pH adjusted to 5.8). 50 L of this PANS was transferred from reservoir of plasmatron system to a PANS chamber tank using water submerged pump. The NS tank was filled with 50 L NS (pH adjusted to 5.8). No solution was added to NS and PANS tanks during the harvest period of 21 days. The plants rotation schedule mentioned above was followed for weeks 1, 2, and 3 (Fig. 35 a, b, c).

2.2.6.2 Treatment 2

For multiple plasma treatments, on day 1, freshly prepared NS and PANS (pH adjusted to 5.8) was transferred to NS and PANS chamber tanks, respectively. Plants were watered using these solutions for week 1. On day 8, solutions from both the tanks were discarded and tanks were cleaned using a scrubber and warm water. Tanks were again filled with NS and PANS prepared on day 8 (pH adjusted to 5.8). The plants were watered using the solutions prepared on day 8 for week 2. On day 15, solutions from both the tanks were discarded again and tanks were thoroughly cleaned. Tanks were then filled with 50 L of freshly prepared NS and PANS (pH adjusted to 5.8) and watered using NS and PANS prepared on day 15 for week 3. The plants rotation schedule mentioned above was followed for weeks 1, 2, and 3 (Fig. 35 a, b, c).

2.2.7 Harvesting

On day 21, all 14 basil plants were harvested from both the chambers. The harvest was done early morning between 8 AM - 10 AM. At the time of the harvest pump, light, and fan were switched off in both the chambers. Five hundred ml of solution was sampled from NS and PANS tanks each and stored in clean plastic bottles for algae, pH, EC, and nutrient analysis. Before harvesting the plants and leaves, their height, number of nodes, branches were measured. For TPC, all the leaves from a randomly selected plant from each chamber were plucked by hand and placed in sterile Whirl-Pak® bag and labelled. For tissue analysis, all the leaves from two randomly selected plants from each chamber were plucked by hand and placed in a Ziploc bag.

One plant was randomly selected from each chamber was cut just above the grodan block and placed in a vacuum oven for dry weight and aroma analysis. From rest of the plants in both the chambers, leaves from 2nd and 3rd nodes from the top were plucked by hand and kept in Ziploc bags for color, texture, and leaf index analysis. After harvesting all the plants, the root length (of the root sticking out of the grodan block) for each plant was measured.

On the same day, the data for CO_2, temperature and relative humidity inside the chambers, and temperature of NS and PANS was collected from CO_2 sensor, temperature and relative humidity sensor, and temperature sensor, respectively.

While handling and touching the plants during the harvest period of 21 days, and during harvesting clean gloves were worn at all times.

2.2.8 Analysis

The following methods were used to analyze the basil samples for Treatment 1 and Treatment 2.

2.2.8.1 Ph, EC, and ORP

The pH of NS and PANS was adjusted to 5.8 using 10 % solution of potassium hydroxide and phosphoric acid 10%. Orion Star™ A111 pH Benchtop Meter from Thermo Fisher Scientific, USA, equipped with Orion™ 9157BNMD Triode™ 3-in-1 pH/ATC Probe from Thermo Fisher Scientific, USA was used to measure the pH. The pH was measured once a day for 21 days. The

ideal pH of solution for basil growth is 5.8. Commercially, growers use pH range of 5.5 - 6. As the

plants start to grow the pH of NS and PANS increased as water evaporated from the tank and

flood table, and plant roots took up more and more nutrients and secreted hydroxyl ions.

Increase in the pH can lead to improper nutrient absorption by plant roots as nutrients such as

calcium form insoluble salts in the solution, limiting its uptake by the roots. When the pH of NS

and/or PANS went above 6, it was adjusted close to 5.8 by using phosphoric acid 10 %.

The EC also tended to go up as water evaporated from the tub and tank. The starting EC

on day 1 was approximately 1.7 mS/cm. The EC was not adjusted but only monitored once every

day using handheld EC meter for both NS and PANS. EC meter was washed properly using

distilled water and wiped dry using Kim wipes. The meter was then dipped in the tanks and held

for 4 s - 5 s before recording the readings.

ORP (oxidation reduction potential) was measured using the same Orion Star™ A111 pH

Benchtop Meter, connected to 9678BNWP Orion™ Metallic Combination Electrode (Redox/ ORP

model with Epoxy body) probe. ORP of the solutions was measured on days 1, 8, 15, and 21.

2.2.8.2 Assay measurement

Assay tests were done on day 1 for Treatment 1, and on days 1, 8, and 15 for Treatment

2. Every assay test was performed in triplicates immediately after the preparation of NS and

PANS. While handling the assay material, eye protection, gloves, and lab coat were worn at all

times.

2.2.8.2.1 Nitrate measurement

For nitrate analysis, Spectroquant® nitrate cell test was performed. 1 ml of NO_3-1K reagent was placed in a reaction cell using a pipette. After that, 0.1 ml of sample was pipetted in the reaction cell. The cell was then closed tightly and contents were thoroughly mixed by shaking the cell for 3 s - 4 s. While doing so the cell was held only by the screw cap because as the contents mix, the cell becomes hot. The cell was then left to stand for exactly 5 min. Immediately after 5 min reaction time, 100 µl of cell contents were pipetted into a clean 96 well microplate. This microplate was placed in the microplate holder of BioTek™ Epoch™ microplate spectrophotometer. The spectrophotometric measurement was taken at 340 nm wavelength to obtain the absorbance reading. A blank reading taken using distilled water was subtracted from the absorbance. The nitrate concentration was then calculated using a standard curve (Fig. 36) obtained by known concentrations of nitrate solution as explained in section 2.1.3.1.

Standard Curve for Nitrates

$y = 0.1362x$
$R^2 = 0.9931$
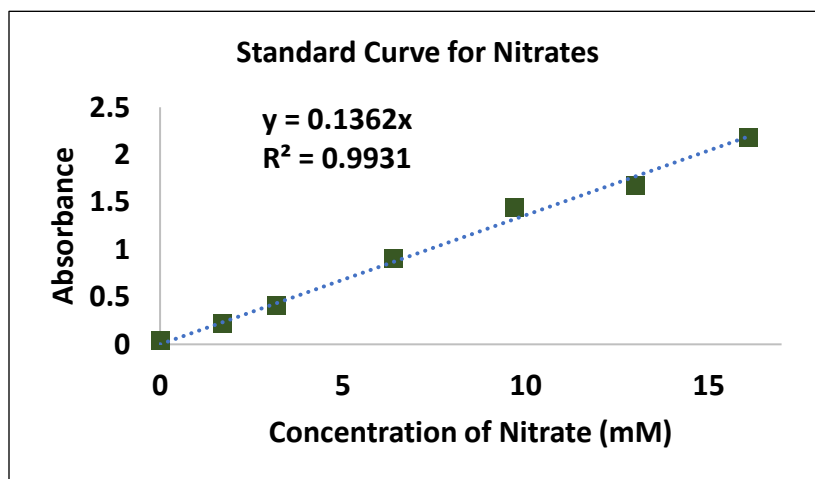
Figure 36: Standard curve for spectrophotometric measurement of nitrates.

2.2.8.2.2 Nitrite measurement

Spectroquant® nitrite cell test was done to measure the nitrite content in NS and PANS. Two microspoons of NO_2-1K reagent was placed into the reaction cell. The microspoon was provided with the assay kit. Eight ml of well mixed sample was then pipetted into the cell. The cell was shaken vigorously by hand for 3 s - 4 s to dissolve the reagent completely and was then left to stand for exactly 20 mins. After the reaction time, care was taken not to shake the reaction cell and 100 µl of cell contents were pipetted in to clean 96 well plate microplate. The absorbance was measured at 451 nm wavelength using BioTek™ Epoch™ microplate spectrophotometer. A blank reading taken using distilled water was subtracted from the absorbance. The nitrite concentration was calculated using a standard curve (Fig. 37) obtained by known concentrations of nitrite solution as explained in section 2.1.3.2.
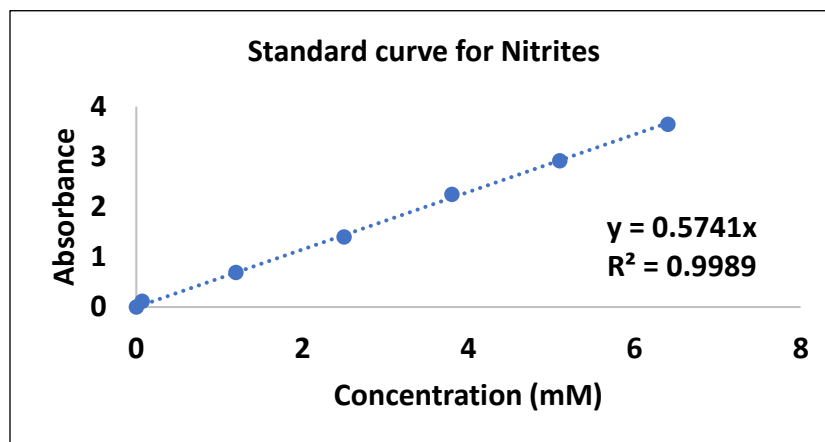
Figure 37: Standard curve for spectrophotometric measurement of nitrites.

2.2.8.2.3 Hydrogen peroxide measurement

Spectroquant® H_2O_2 cell test was performed to measure hydrogen peroxide present in NS and PANS. 10 ml of sample was pipetted into a reaction cell containing sulfuric acid (0.5 mol/l Titripur®), then the cell was closed tightly and shaken by hand for 3 s - 4 s. The cell was then left to stand for exact 2 min. After 2 min reaction time, 100 µl of the cell contents were pipetted into 96 well microplate and absorbance were measured at 410 nm wavelength using BioTek™ Epoch™ microplate spectrophotometer. A blank reading taken using distilled water was subtracted from the absorbance. The H_2O_2 concentration was calculated using a standard curve (Fig. 38) obtained by using known concentration of hydrogen peroxide solution as explained in section 2.1.3.3.
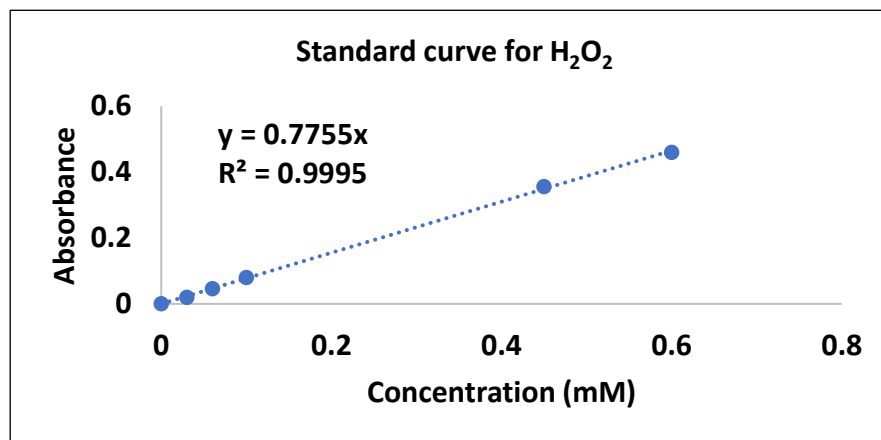
Figure 38: Standard curve for spectrophotometric measurement of hydrogen peroxide.

2.2.8.3 Test strips measurement nitrate, nitrite, and hydrogen peroxide

Test strips were used as an easy and quick method to quantify nitrate, nitrite, and hydrogen peroxide concentration of NS and PANS. This measurement was performed on day 1 for Treatment 1 and on days 1,8, and 15 for Treatment 2. The test strip measurement was done in triplicates immediately after the preparation of NS and PANS. These measurements were done in addition to assay tests as a quick and easy method for determination of reactive species.

2.2.8.3.1 Nitrate measurement

The nitrate measurement strip was dipped in the sample solution taken in a beaker for 1 s. Care was taken not to shake the excess solution from the strip. The strip was held pad side up for approximately 30 s and then the developed color on the pad was compared to the color chart given by Insta-Test® to obtain nitrate content in ppm. If the pad color fell between 2 color blocks from the reference chart then the test results were estimated to match the closer color value.

2.2.8.3.2 Nitrite measurement

MQuant® nitrite test strips were dipped for 1 s in a sample solution taken in a beaker. The nitrite strip was left to stand for approximately 15 s. The developed color on the test pad was then compared to color chart provided by MQuant® to get nitrite content in ppm.

2.2.8.3.3 Hydrogen peroxide measurement

Bartovation test strip was dipped in a sample solution for 1 s. The excess liquid was removed from the strip by shaking it 2 - 3 times by hand. The strip was then left to stand for approximately 10 s. The color developed on the test pad was then compared to the color scale given by Bartovation to obtain hydrogen peroxide concentration in ppm.

2.2.8.4 Morphological analysis

2.2.8.4.1 Plant height

On day 1, height of all the seedlings was measured using a ruler after transferring them to NS and PANS chambers. The measurement was done between the top of grodan block and top of the seedling, and was recorded in centimeters. The height measurement was done on every 3rd day (days 1, 3, 6, 9, 12, 15, 18, and 21) till the harvest day.

2.2.8.4.2 Number of branches, number of nodes, and root length

Branches and nodes represent plant growth. Number of branches were counted on day 21 just before harvesting the basil plants. A plant stem is divided into nodes and internodes. A node is a small growth area on the stem from where leaves, branches grow out. An internode is

an area between two consecutive nodes. The plant nodes were counted on day 21 before

harvesting the plants. Plant roots were observed almost every day, to look for any development

of root disease. The plant root length was measured on day 21 after harvesting the plants using

a ruler. Each grodan block was lifted up by hand to measure the root length (length of the root

sticking out of the grodan block).

2.2.8.4.3 Leaf index (LI)

Leaf index is a measure of leaf area. On day 21, leaves were randomly plucked by hand

from 2nd and 3rd nodes from top and placed in a clean Ziploc bag. From the bag, 10 leaves were

randomly selected for leaf index measurement. The length (L) of each leaf from the base to the

tip of the leaf was measured using a ruler (Fig. 39). The width (W) of each leaf (the distance

between the two farthest points on the leaf edge) was measured using a ruler (Fig. 39).
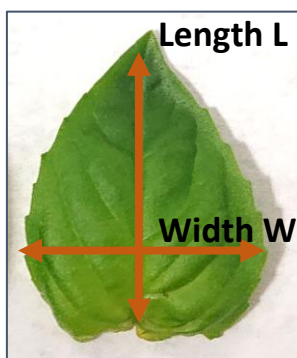
Figure 39: Basil leaf length L and width W.

The leaf index was then calculated for each leaf by using the following formula-

LI (cm^2) = L x W x 0.8 (Cochran and Fulcher, 2013)   Eq. 1

2.2.8.5 Colorimetric analysis

To determine the color of plant leaves and the difference in the color of NS and PANS

treated leaves, basil leaf color was determined in terms of L*, a*, and b* values using Konica

Minolta CR-410 colorimeter. On day 21, leaves from 2nd and 3rd nodes were randomly plucked by

hand and placed in a clean Ziploc bag. From the bag, 10 leaves were selected randomly. Each

leaf was then placed flat on an opaque white base plate. The color values were measured at 3

random locations (the leaves were bigger than the colorimeter probe) on the leaf using the

colorimeter (Fig. 40). Average values for L*, a*, and b* were calculated.

Figure 40: Basil leaf placed on an opaque white plate and its color being measured using Konica

Minolta CR-410 colorimeter.

To analyze the difference in the color of NS and PANS leaves, ΔE_{ab}^* was calculated. ΔE_{ab}^* is the numerical value for color difference and was calculated against color values for basil leaves grown using NS. Greater the ΔE_{ab}^* value greater is the difference in the color of NS and PANS leaves. ΔE_{ab}^* was calculated using the following formula-

$$\Delta E_{ab}^* = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2}$$ Eq. 2

2.2.8.6 Texture analysis

2.2.8.6.1 Puncture test

For texture analysis of basil leaves, puncture test was performed using Brookfield CT-3 texture analyzer. A 2 mm diameter cylindrical probe was used for this test. For performing the puncture test, a basil leaf was held between two concentric circular plastic plates and a washer as depicted in Fig. 41.
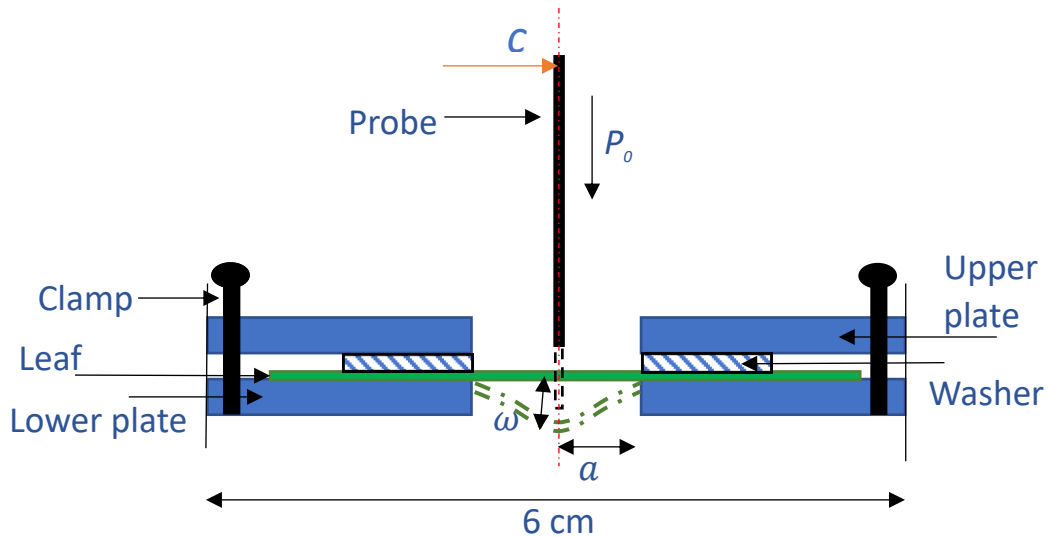
Figure 41: Depiction of basil leaf held between two plastic plates and a washer being punctured by a 2 mm diameter probe with radius c, radius of leaf area exposed a, maximum deflection at the center ω (figure not up to scale).

Out of the two plastic plates, the bottom plate was fixed to the metal base and the distance between the plates was adjustable such that the top plate could be taken up to place the basil leaf between the plates and then the leaf was sandwiched between the plates by using clamps. The top plate had a washer glued to it which aided to gently press the leaf at the time of its puncture (Fig. 42 a, b, c). This modification helped to hold the leaf in place during the puncture test without physically holding and stretching it.
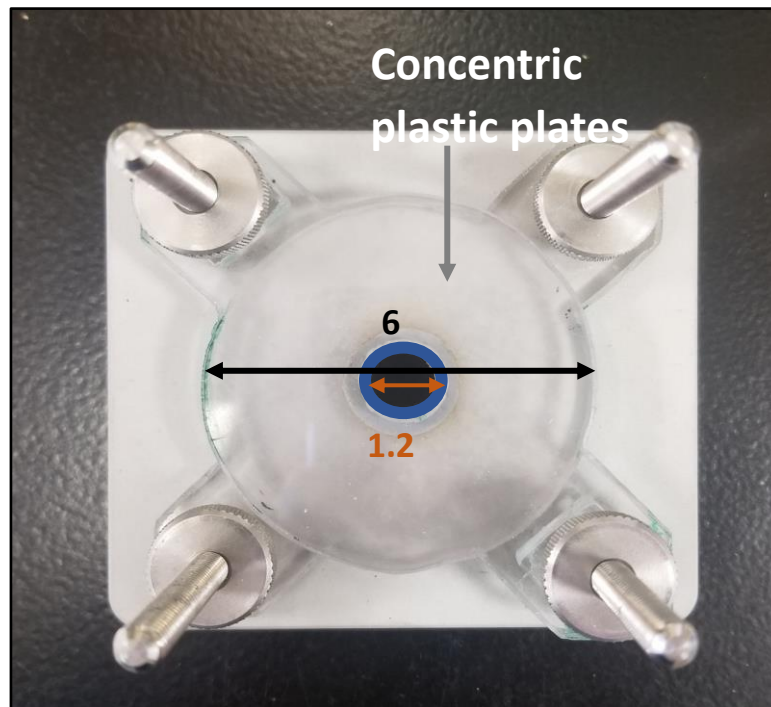
(a)
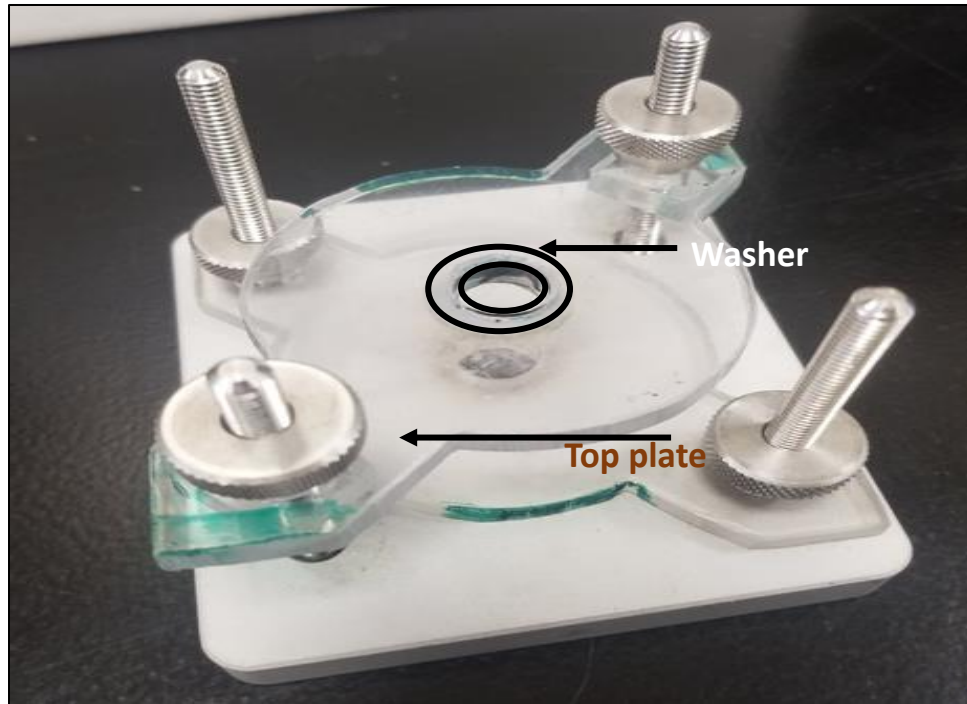
(b)
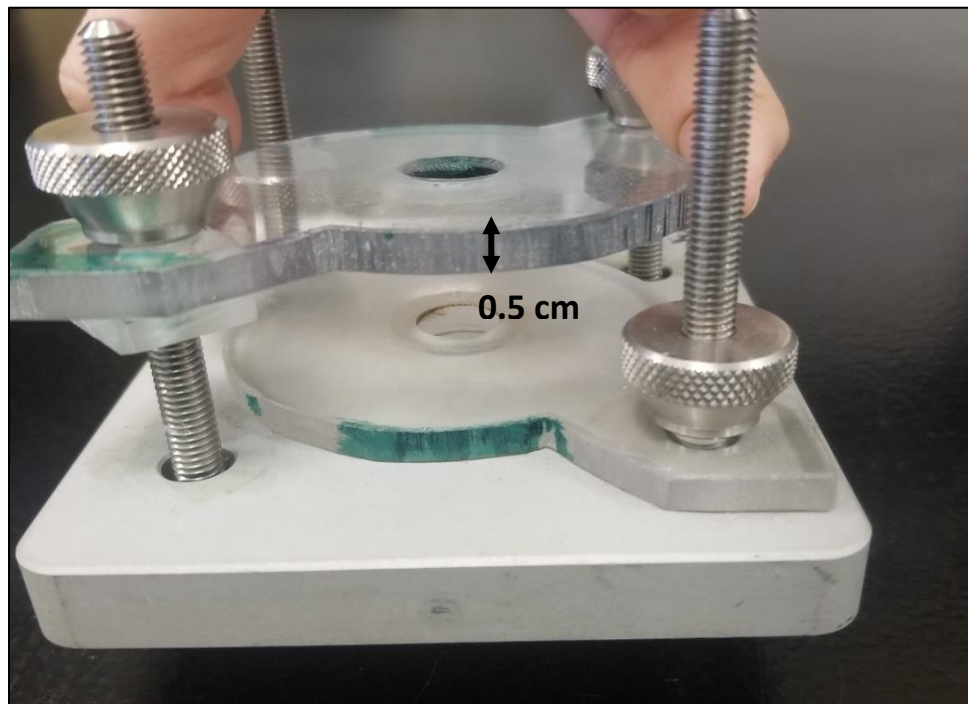
(c)

Figure 42: (a) Top view of the modified base plate for puncture test of basil leaves with two concentric circular plastic plates with 6 cm diameter (b) Washer attached to the upper plate from the inside (circled area) to gently press the basil leaf, (c) Adjustable plastic plates of 0.5 cm thickness attached to the base plate to hold the basil leaf flat during the puncture test.

2.2.8.6.1.1 Peak force measurement

Peak force required to puncture one basil leaf was obtained after performing the puncture test. Leaves were randomly plucked by hand from 2nd and 3rd nodes of the plants and were placed in a clean Ziploc bag. From the bag, 10 leaves were randomly selected for the puncture test. Only one leaf was used at a time. Puncture test was carried out on all 10 leaves.

(a)   (b)
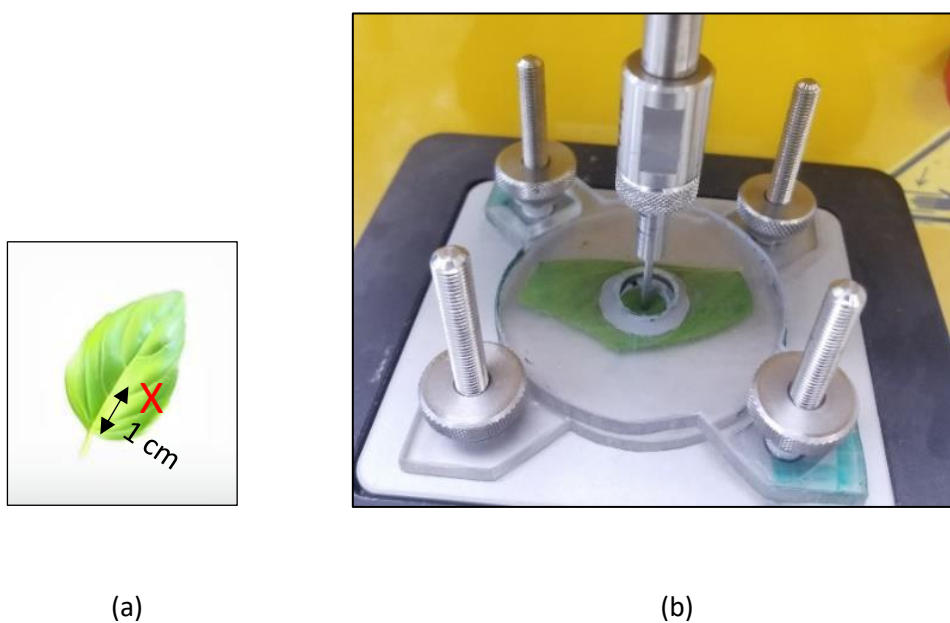
Figure 43: (a) Approximate location of the puncture test on a leaf, (b) Puncture test being performed on the basil leaf using Brookfield CT-3 texture analyzer and 2 mm cylindrical probe at a test speed of 1 mm/s.

Each leaf was then placed between a modified base plate (explained in 2.2.8.6.1) and puncture test was performed (Fig. 43 a and 43 b) at 1 mm/s test speed of the probe using Brookfield CT-3 texture analyzer. The peak force required to puncture the leaf was noted and a graph of force vs distance was obtained.

2.2.8.6.2 Leaf toughness

The graph obtained from the puncture test of basil leaves was further used to calculate area under the force vs distance curve. This area was used to estimate the basil leaf toughness (Gutiérrez-Rodríguez et al., 2013).

2.2.8.6.3 Young's modulus measurement

To quantify the difference in the leaf elasticity of NS and PANS basil leaves, Young's modulus value was estimated using the following formula (Ugural, 2017).

$$\omega = 3 \; \frac{(1-v)}{4} \; \frac{P_0 \; C^2}{E \, t^2} (a^2 - \frac{3}{4}c^2 - c^2 \ln\frac{a}{C}) \qquad \text{Eq. 3}$$

where

ω – Maximum deflection at the center (m), v – Poisson's ratio for a plant leaf = 0.25 Saito et al. (2006), P_0 – Pressure (N/m^2), c – Radius of the probe (m), E – Young's modulus (N/m^2), t – Thickness of the leaf (m), a – Radius of the leaf area exposed (m)

2.2.8.7 Algae analysis

For Treatment 1 and Treatment 2, solution samples from NS and PANS tanks were collected on days 1, 8, 15, and 21 in clean glass bottles. The bottle was shaken by hand for 3 s - 4 s before pipetting 2 ml solution from it. This pipetted solution was placed in a clean glass tube. Two drops of Lugol's solution was then added to the glass tube with the help of a dropper. The glass tube was left to stand for 10 min to allow Lugol's solution to stain the algae. After 10 min, the glass tube was vortexed for 2 s and 1 ml solution was pipetted into the Sedgewick cell chamber. While doing so a glass cover slide was kept on top of the chamber in a slanting way to allow air to escape and minimize the formation of air bubbles. The solution was pipetted through a small gap between the cover slide and the edge of the chamber (Fig. 44).
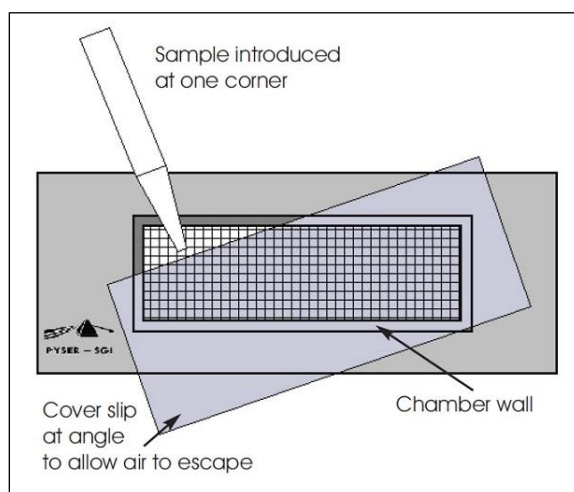
Figure 44: Sedgewick cell with a slanting cover slide placed on the top.

After placing the solution in the cell chamber, it was left to stand for 10 min to allow algae cells to settle down. Then the Sedgewick cell was placed under a microscope (Olympus BH-2 BHSP light microscope) and algae cells were counted using 10x objective. The Sedgewick cell chamber consisted of 1000 small squares each corresponding to 1 mm^3. To calculate total

algae content, algae cells present in 50 random small squares were counted and average

number was calculated using Equation 4. This test was performed in triplicates.

Total algae cells/ml of solution = Average algae cells in each square x 1000　　　　　Eq. 4

2.2.8.8 Microbial analysis

On day 21, all the leaves from one random plant in each chamber were plucked by hand

using clean gloves and placed in a sterile Whirl-Pak® bag. 14 - 17 leaves weighing approximately

25 g were randomly selected from the plucked leaves and placed in a sterile Whirl-Pak® bag

containing 225 ml of peptone water. This bag was put in a stomacher for 1 minute. 1 ml of

aliquot pipetted from the stomached bag was then transferred to 9 ml sterile peptone water in

a glass tube (10^1 dilution). Further, four such dilutions (10^2, 10^3, 10^4, 10^5) were made by

transferring 1 ml aliquot from the previous dilution to 9 ml sterile peptone water. 0.1 ml of each

serial dilution was then plated onto total plate count agar plates in duplicates. Plates were

placed in an incubator at 37 °C for 24 h and then the microbial colonies were counted. To

calculate the aerobic total plate count following formula was used:

CFU (colony forming units)/g of the sample = (no. of colonies) x (dilution factor) / (volume
plated)　　　　　　　　　　　　　　　　　　　　　　　　　　　　　　Eq. 5

2.2.8.9 Dry weight analysis

On day 21, plants were harvested by cutting the stems just above the surface of a grodan block using scissors. The cut plants were weighed using a weighing balance and their fresh weight was noted. Cut plants were then placed inside a vacuum oven at 40 °C for 10 h - 12 h until constant weight was obtained. Plants were then weighed and their dry weight was noted. The moisture content of the basil plants was calculated using the following formula:

% Moisture = (Fresh weight of sample – Dry weight of sample) x 100 / (Fresh weight of sample)

Eq. 6

2.2.8.10 Aroma analysis

Aroma analysis was done to the estimate essential oil profile of the basil grown using NS and PANS. The essential oil content and profile is affected by plant nutrition and environmental conditions. Thus, to see if there is any change in the aroma of basil this analysis was performed. After the dry weight analysis, the same dried sample (section 2.2.8.9) was further used to determine aroma compounds. The analysis was performed at Rutgers Plant Biology Department in by Lara Brindisi in Dr. Jim Simon's laboratory. The GCMS (gas chromatography mass spectrometry) protocol for aroma analysis is given below (Deschamps et al., 2008).

Five hundred (500) mg of dried basil sample was mixed with 5 ml Tert-Butyl Methyl Ether in a glass vial and then was kept under cool conditions for 24 h. The contents were then transferred to 1.5 ml Eppendorf tube containing a small amount (~50 mg of sodium sulfate) and then centrifuged for 2 min at high speed. The supernatant was transferred to 1.5 ml GC vials.

Further chromatographic analysis was done using Shimadzu 2010 Plus gas chromatograph

equipped with an AOC-6000 auto-sampler. The relative abundance analysis of compound

fragments was done on Shimadzu TQ8040 MS. An injection volume of 1 µl was separated using

chromatographic grade helium on a H-Rxi-5Sil MS column held at 35 °C for 4 min then heated to

250 °C at 20 °C/min then held for 1.25 min at 250 °C. GCMS solution v4.3$^©$ software from

Shimadzu Corporation was used to determine peak integration percentages. Individual

compound identifications were made by matching Kovats index values (Jennings and Shibamoto,

1980) to the literature and by comparison of mass spectra with a mass spectral library.

2.2.8.11 Tissue analysis

To compare the quality of basil grown using NS and PANS, it was important to evaluate

the nutrient profile of the basil samples. This was determined using tissue analysis of fresh basil

samples. Fresh samples were taken by handpicking all the leaves from one random plant on day

21. The leaves were kept in a Ziploc bag and shipped on the same day to North Carolina State

University, Raleigh, NC. The tissue analysis was performed at NCDA (North Carolina State

Department of Agriculture), Agronomic division.

To quantify major elements (N, P, K, Ca, Mg, and S) and minor elements (Fe, Cu, Mn, B,

and Zn) in the plant tissue, ICP emission spectroscopic method was used (Plank, 1992).

2.2.8.12 Nutrient analysis of NS and PANS

To evaluate the nutrients, present in NS and PANS, the nutrient analysis of the solutions was done. Freshly prepared 250 ml each of NS and PANS samples were shipped on day 1 (for Treatment 1) and on days 1, 8, 15 (for Treatment 2) to North Carolina State University, Raleigh. The nutrient analysis was performed at NCDA (North Carolina State Department of Agriculture), Agronomic division according to method given by Plank 1992.

2.2.9 System disinfection

On day 21, after harvesting basil plants, both the flood tables and tanks were thoroughly cleaned. First, the flood table was washed with warm water and then cleaned using a scrubber. The scrubber helped to remove algae layer from the flood tables. Tanks were cleaned using warm water and scrubber. After visual inspection of flood tables and tanks to make sure no debris and/or algae is present on the surface, they were washed with OxiDate® 2.0 (1:100 solution). The pump was then soaked in OxiDate® 2.0 (1:100 solution) overnight. The OxiDate® 2.0 solution was used to get rid of any residual algae in the system. The chambers and tanks were kept open overnight to allow them to dry.

2.2.10 Energy measurement of energy consumed by each chamber to grow basil

The energy (kWh) consumed by the light, plasmatron, pump, and fan was estimated using a wattmeter. The light and fan were plugged onto the wattmeter for 24 h to obtain the

consumed energy and then estimate the energy used by them in 21 days (light was kept on for 16 h per day, fan was always on for both the treatments). The plasmatron system was plugged onto the wattmeter for a single run (pH drop to 3.5) to obtain the energy consumed for Treatment 1, then was multiplied by 3 to obtain the energy consumed by the plasmatron system for Treatment 2. The pump was plugged onto the wattmeter for 1 h to estimate the total energy consumed by it for 21 days (pump was switched on 8 times a day for 8 min for both the treatments). To obtain total energy consumption (kWh) by the PANS chamber to grow the basil plants for 21 days, the energy consumed by the light, plasmatron system, fan, and pump was added. From this estimated amount the energy used by plasmatron system was subtracted to find the total energy consumed by NS chamber to grow the basil plants for 21 days.

2.2.11 Estimation of the % water used by the basil plants to grow for 3-week harvest period

The solution quantity at the start of each treatment was 50 L (initial amount). The solution that was left at the end of 21 days was measured (left amount). The subtraction of these two gave us solution that remained unused. Out of the used solution, the solution that was actually used by the basil plants was equal to water present in the plants on day 21. This was estimated using the fresh weight and moisture content of the plant. Thus, the % water used by basil plants to grow for 21 days was obtained using the following formula.

% water consumed by the plants = (Fresh weight * Moisture content of basil plants)/Initial

amount of solution taken * 100   Eq. 7

2.2.12 Statistical analysis

Statistical analysis was done to evaluate the statistical significance between the differences of the means of NS and PANS basil samples. The analysis was done using One-way analysis of the variance (ANOVA) in Microsoft® Excel® 2015. This analysis was used to check whether the level of significance (p value) was less than 0.05. In that case the null hypothesis was rejected.

3. RESULTS AND DISCUSSIONS

In this section results for Treatment 1 (single plasma treatment, plants watered using nutrient solution (NST1) and plasma activated nutrient solution (PANST1)) and Treatment 2 (multiple plasma treatments, plants watered using nutrient solution (NST2) and plasma activated nutrient solution (PANST2)) will be presented and discussed in section 3.1 and section 3.2, respectively. After that to see which plasma treatment was better, comparison of two plasma treatments (PANST1 and PANST2) will be discussed in section 3.3. In the end, to evaluate and understand how these two plasma treatments faired with respect to control (NST1 + NST2), results for the control will be compared with the two plasma treatments in section 3.4.

3.1 Treatment 1

In this section results for basil growth and analysis using single plasma treatment (refer to section 2.2.6.1) are presented and discussed. Rutgers Devotion DMR basil seedlings were grown using NS as explained in section 2.2.3. On the 14th day the seedlings (Fig. 45) were transferred to NS (Fig. 46 a) and PANS (Fig. 46 b) chambers and were watered as per the schedule described in 2.2.6.1 using NS (NST1 for Treatment 1) and PANS (PANST1 for Treatment 1), respectively for next 21 days. On day 21, all the plants were harvested (Figs. 46 c and 46 d) and their growth and quality parameters were evaluated following the methods explained in section 2.2.8. Two batches of basil plants were grown using NST1 and PANST1 for single plasma treatment variation (Treatment 1, n=3).

The average chamber temperature for NS and PANS chambers was (23.4 ± 5.1) °C and (23.3 ± 0.5) °C, respectively, relative humidity for NS and PANS chambers was (34.5 ± 3.7) % and (33.7 ± 4.1) %, respectively, and CO_2 concentration for NS and PANS chambers were (575.1 ± 20) ppm and (580.3 ± 9.1) ppm, respectively. The average light intensity on top of the plants was approximately 250 µmol/m^{2}s in both the chambers.

Figure 45: Basil seedlings ready for transferring to NS and PANS chambers after 14 days.

(a)

(b)

(c)

(d)

Figure 46: Growth of basil plants during harvest period of 21 days for Treatment 1: (a) basil plants in NS chamber on day 1, (b) basil plants in PANS chamber on day 1, (c) basil plants in NS chamber on day 21, (d) basil plants in PANS chamber on day 21.

3.1.1 Characterization of NST1 and PANST1

3.1.1.1 pH, EC, and ORP of NST1 and PANST1

NST1 and PANST1 were prepared on day 1 of the harvest period. On day 1, the pH values of freshly prepared NST1 and PANST1 were 6.81 ± 0.04 and 3.63 ± 0.1, respectively, which were adjusted to 5.8 and 5.81, respectively, by using potassium hydroxide 10 % solution. As plants grow, they take up more and more nutrients leading to unbalanced absorption of cations and anions, and secret hydroxyl ions from their roots (Resh, 1995). This led to an increase in the pH of NST1 and PANST1 over time. Such increase can lead to improper nutrient absorption due to change in the solubility of some micro nutrients and some macro nutrients such as calcium by plant roots (Resh, 1995; Sonneveld and Voogt, 2009). Hence, pH was adjusted every time it reached 6 or above. The pH had to be adjusted to 5.8 on days 4, 7, 11, 15, and 19, as it went above 6, as seen in Fig. 47. On day 21 the pH values of NST1 and that of PANST1 were 5.96 ± 0.01 and 6.04 ± 0.06, respectively.
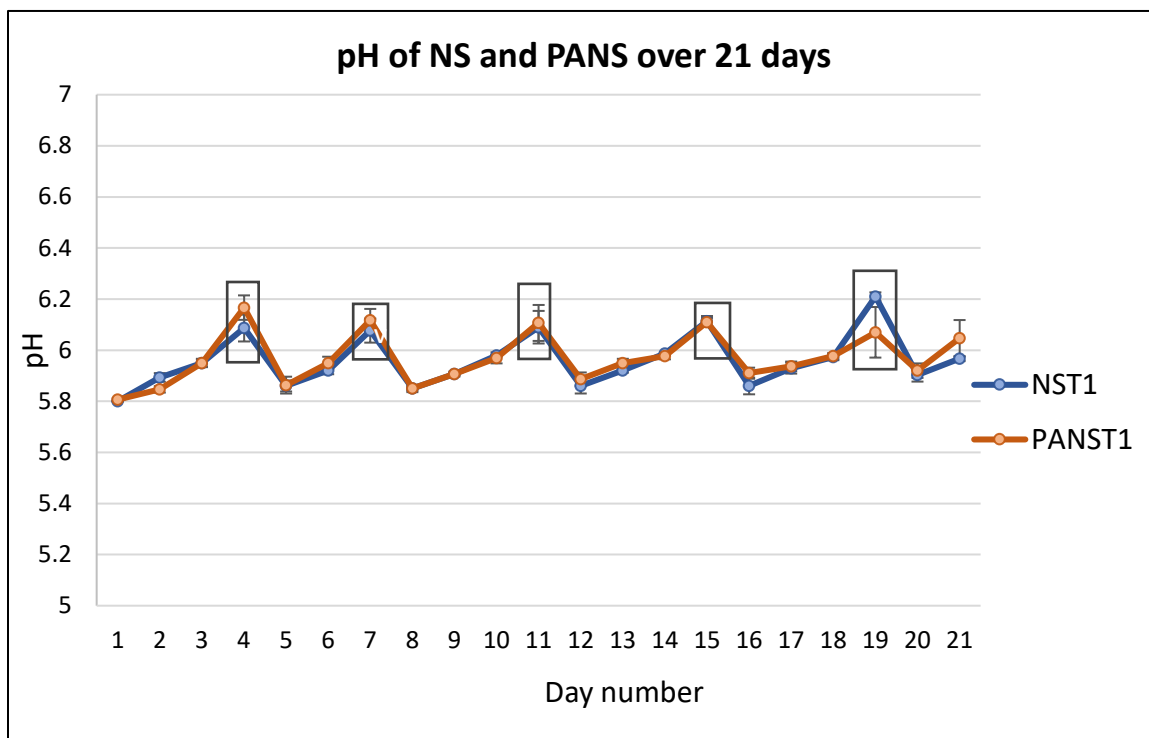
Figure 47: Variation of pH of NST1 and PANST1 over harvest period of 21 days and pH

adjustment on days 4, 7, 11, 15, and 19. (error bars indicate standard deviation, n = 9).

Growing conditions and type of crop determine the optimum EC of NS for plant growth

(Sonneveld and Voogt, 2009). The optimum EC for hydroponic basil growth has been reported to

be 1.7 mS/cm. The half strength nutrient solution for seedling growth had an EC of (0.8 ± 0.5)

mS/cm. After transferring the seedlings to the chambers, NST1 and PANST1 were used to water

the plants and their EC was monitored every day throughout the harvest period (Fig. 48).

Generally, in closed hydroponic systems the NS used for plant growth is not recirculated for

more than 2 – 3 weeks due to its high EC value which affects proper nutrient uptake by the

plants. As plants start to grow, they absorb relatively more water than salts leading to increase

in the EC of NS (Resh, 1995). Also, there was water evaporation from the flood table. This led to

increase in the EC of NST1 and PANST1 over the period of 21 days as seen the in Fig. 48.
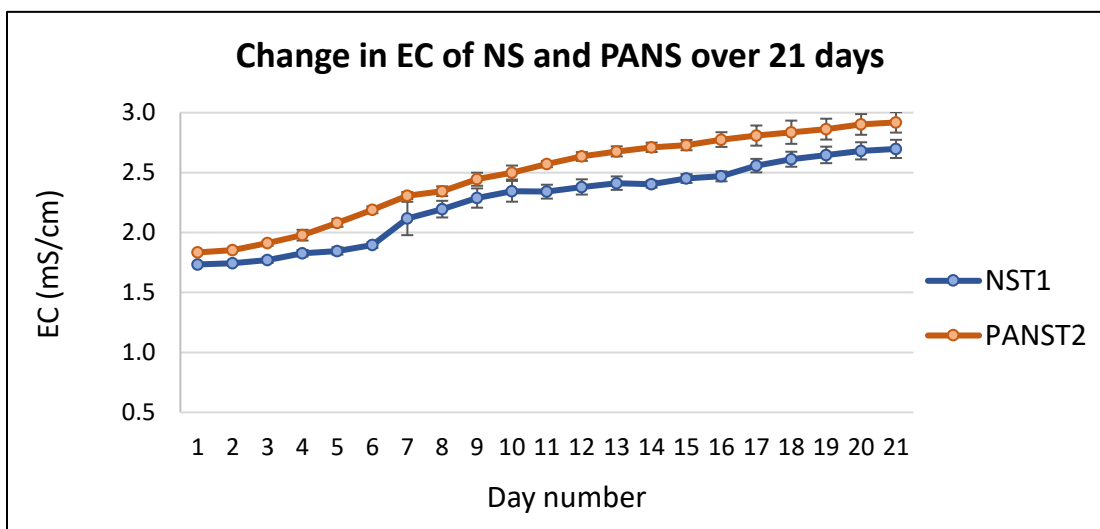
Figure 48: Variation of EC of NST1 and PANST1 over the harvest period of 21 days. (error bars

indicate standard deviation, n = 9).

The starting values of EC for NST1 and PANST1 on day 1 were 1.734 mS/cm, 1.834

mS/cm, respectively, which increased to (2.69 ± 0.08) mS/cm and (2.91 ± 0.08) mS/cm,

respectively, on day 21. Throughout the harvest period of 21 days, higher EC values for PANST1

as compared to NST1 were noted, as seen in Fig. 48, due to increased total nitrogen. This was

also supported by the nutrient analysis data discussed in section 3.1.10.

The ORP value of NST1 and PANST1 was measured at the end of every week. The ORP

value of PANST1 could be related to its antimicrobial potential due to the presence RONS (Al-

Haq et al., 2005). The ORP value of PANST1 was significantly higher $(p<0.05)$ than that of NST1

on day 1 and on day 8 (Fig. 49). No significant difference was seen in their ORP values after day 8

(Fig. 49). This implied that, the PANST1 had more oxidizing power in the first week due to presence of reactive species which could aid in algae removal from PANST1 and could provide more nitrogen to the basil plants.
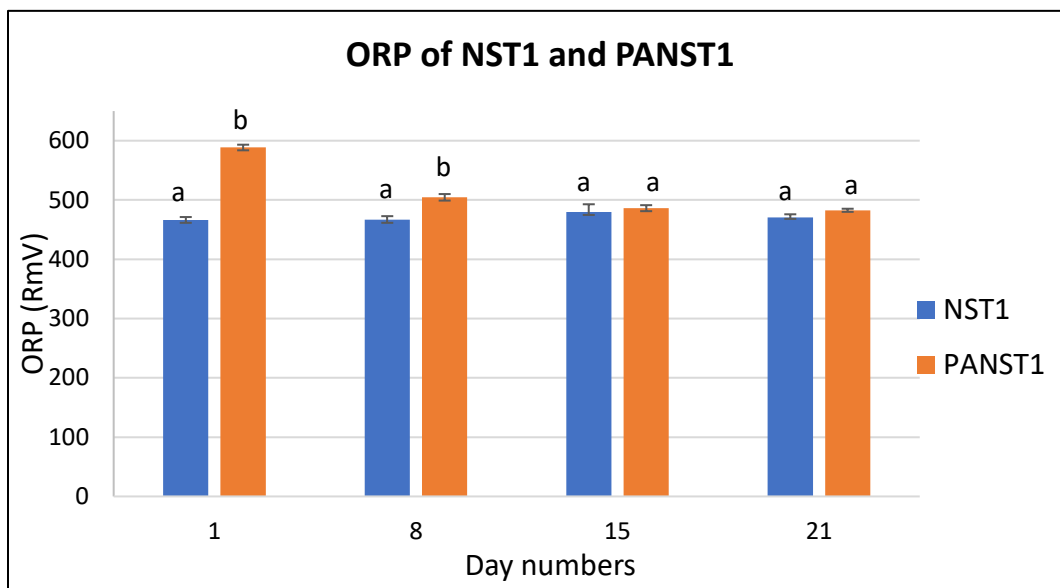
Figure 49: Change in the ORP of NST1 and PANST1 over the harvest period of 21 days, (error bars indicate standard deviation, n = 9, data not sharing the same letter are significantly different from each other, one-way ANOVA, $p<0.05$).

3.1.1.2 Nitrates, nitrites, and hydrogen peroxide

To investigate the effect of plasma treatment on PANST1 and to evaluate the difference between NST1 and PANST1, reactive species concentration was measured. RONS species especially nitrates and nitrites can be beneficial to plant growth and can also play a part in enhancing the microbial quality of hydroponic solution discussed in section 1.3.4.2.1 and 1.3.4.1, respectively. Thus, it was important to estimate their concentration in NST1 and PANST1. This was done using assays and test strips.

The assay tests were done immediately after preparation of NST1 and PANST1 on day 1 of the harvest period (Table 14). After plasma treatment, the nitrate and nitrite concentrations increased by (40 ± 4.7) ppm and (9.1 ± 2.6) ppm, respectively. Hydrogen peroxide was absent in NST1 and PANST1. The absence of hydrogen peroxide in the PANST1 could be due to inverse correlation of its generation with the EC of the solution causing photolysis reactions (Luke et al., 2008; Kirkpatrick et al., 2005). Also, according to Burlica et al. (2006) the presence of nitrates can interfere with hydrogen formation. In their study, no hydrogen peroxide was detected in water treated with gliding arc plasma with air as a gas.

Table 14: Nitrates and nitrites concentration on day 1 measured using assays for Treatment 1

Characteristic	NST1	PANST1
Nitrates (ppm)	151.9 ± 3.3	191.9 ± 3.1
Nitrites (ppm)	9.6 ± 1.6	18.8 ± 1.9

The RONS concentration was also measured using test strips as it was a quick method to estimate their concentration. This was done immediately after the preparation of NST1 and PANST1 on day 1 (Table 15). The data obtained from test strips were consistent with the assay measurements. The nitrates and nitrites concentration of PANST1 increased by 25 % and 100 %, respectively. Hydrogen peroxide was not detected using test strips as well.

Table 15: Nitrates and nitrites concentration on day 1 measured using test strip for Treatment 1

Characteristic	NST1	PANST1
Nitrates (ppm)	160	200
Nitrites (ppm)	10	20

3.1.2 Morphological analysis

3.1.2.1 Plant height analysis

The seedlings for NS and PANS chambers were grown for 14 days in controlled environment of 25 °C, at 75% relative humidity, and 100 µmol/m^{2}s PPFD. They were then further grown for 21 days in enclosed chambers. Height of the seedlings was measured on day 1 after transferring them to NS and PANS chambers. The height of each plant was then measured on every third day till the harvest day. Plant height is a characteristic of plant growth and depends on growth environment and nutrient availability (Resh, 1995). On day 1, height of seedlings for NS and PANS chambers was (4.14 ± 0.46) cm and (4.36 ± 0.36) cm, respectively, and it was not significantly different from each other (p>0.05). Thus, any subsequent differences in the plant heights can be attributed to the use of PANST1 to water the plants. Plant heights for NS and PANS chambers were not significantly different on days 1, 3, 6, 9, and 12 (p>0.05). But on days 15, 18, and 21 a significant difference (p<0.05) was found between the plant heights of NS (10.9 ± 0.05, 14.7 ± 0.3, 17.6 ± 0.4) cm and PANS (11.9 ± 0.4, 15.9 ± 0.4, 19.2 ± 0.4) cm chambers, with the height of PANS plants being significantly (8 % - 9 %) more (Fig. 50). This

could be because of use of PANST1 to water the plants. The PANST1 had more total nitrogen and nitrate on day 1 and also throughout the harvest period as seen from its increased EC and ORP value. This was also supported by the nutrient analysis of PANST1 and NST1 (discussed in section 3.1.10) on day 1 and 21.

Nitrogen is the most limiting nutrient for plant growth. It is necessary for synthesis of amino acids and nucleic acids (Wiedenhoeft, 2006). The above ground growth, that is the stems and the leaves of a plant are affected by the amount of nitrogen available for absorption, most of which is absorbed in the form of nitrates (Rorabaugh, 2002). In PANST1 on day 1, the nitrate, nitrite concentrations were approximately higher by 40 ppm and 10 ppm, respectively, than the NST1. This resulted in increased total nitrogen in PANST1. On day 21, nitrate concentration was more in PANST1 by (23.5 ± 10.5) ppm than NST1 as determined by the nutrient analysis of the solutions. Thus, throughout the harvest period, total nitrogen content was more in PANST1 than NST1, which could have led to significant increase in basil plant height grown using PANST1. However, researchers have not able to directly relate increase in basil plant height with nitrogen.
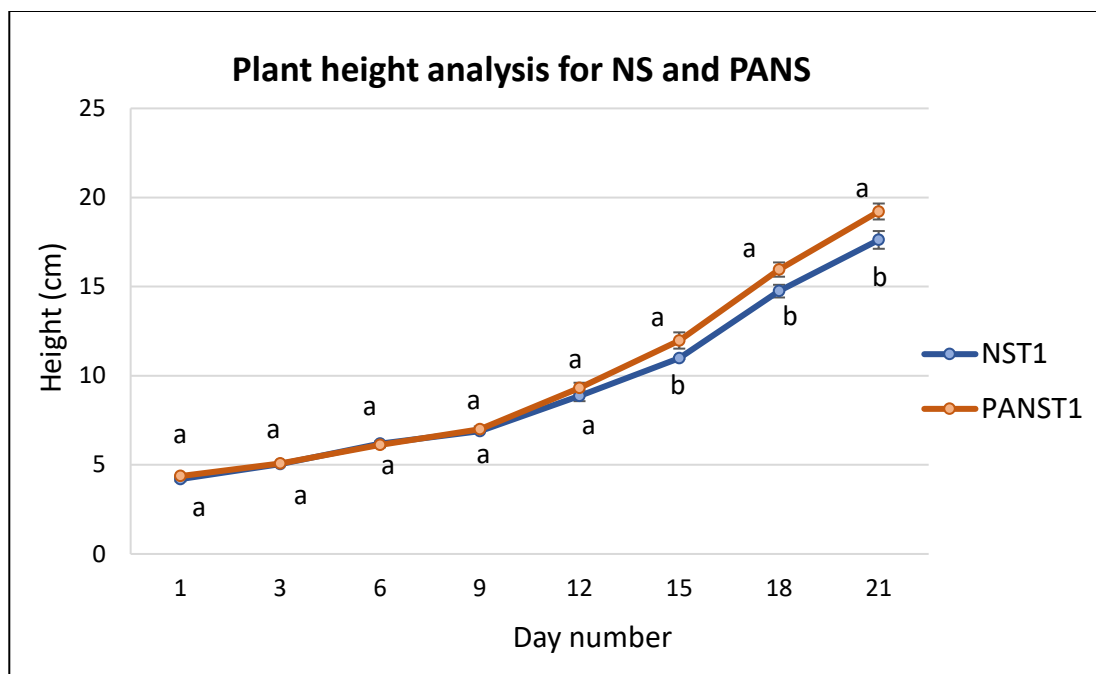
Figure 50: Plant height analysis of basil plants for Treatment 1 over the harvest period of 21 days, (error bars indicate standard deviation, n = 42, data not sharing the same letter on a given day are significantly different from each other, one-way ANOVA, $p<0.05$).

Nurzyńska-Wierdak et al. (2011 a) studied the effect of nitrogen foliar feeding and non-feeding on four basil cultivars (Kasia, Wala, Genua-star, and Opal) grown in a medium silty loam soil and found that nitrogen foliar feeding (0.5 % urea solution) led to significant increase in the plant height in all the cultivars. This was attributed to direct absorption of nitrogen forms through the leaves. In another study, Nurzyńska-Wierdak et al. (2012) reported significant decrease in the height of Kasia, Wala, and Genua-Star with increase in nitrogen dose (ammonium nitrate) from (0.2 – 0.9) g.dm^3. Sifola and Barbieri (2006) reported no significant difference in the heights of three different basil cultivars grown in the field using nitrogen fertilization (ammonium nitrate) from (0, 100, and 300) kg ha^{-1}. In hydroponic experiments done using nutrient flow technique (NFT) on different basil cultivars to see the effect of increasing EC

(0.5 – 4 mS/cm) on basil morphology, Walter and Currey (2018) did not find any significant effect of EC on basil plant morphology. Thus, according to the previous research an increase in the nitrogen dose does not directly correlate to an increase in the basil plant height.

The research on basil plant morphology and nitrogen focuses on a fixed nutrient concentration at the start of the harvest period. However, as the plants grow they take up more and more nutrients resulting in less available nutrients in the soil. This was also true for hydroponic experiments done by Water and Currey (2018). In their experiments, the increase in the EC was achieved by increasing the total nutrient content of the water. However, in this research the higher EC of PANST1 was due to increased total nitrogen only. Also, unlike literature, EC of the NST1 and PANST1 increased over time. Thus, the growing condition of basil plants were not similar to the literature which could have affected the basil plant height.

3.1.2.2 Branches, nodes, and plant root length analysis

Number of branches and nodes of basil plants were noted on day 21. Plant roots were observed almost every day and their lengths were measured on day 21.

Table 16: Number of branches, nodes, and root lengths for plants grown using NST1 and PANST1

Characteristic	NST1	PANST1
Number of branches	9.1 ± 0.9[a]	9.3 ± 0.9[a]
Number of nodes	3.5 ± 0.1 [a]	3.4 ± 0.2 [a]

Root length (cm)	15.2 ± 0.8 [a]	13.5 ± 0.7 [b]

(n = 42, data indicating different superscript letters across the columns in the same row are significantly different, one-way ANOVA, p<0.05)

The number of nodes of a plant determine the number of branches for a plant. Nodes are a small growth area on the stem from where the leaves and branches grow. In Treatment 1, there was no significant difference (p>0.05) in the number of nodes in basil plants grown using NST1 and PANST1 which explains the lack of any significant difference (p>0.05) in number of branches for basil plants grown using NST1 and PANST1 as seen in the Table 16.

Results for the effect of increased nitrogen on basil plant morphological characteristics such as branching and nodes are not very direct (Nurzyńska-Wierdak et al., 2012; Nurzyńska-Wierdak 2011; Sifola and Barbieri, 2006). Nurzyńska-Wierdak et al. (2012) grew three different basil cultivars (Kasia, Wala, and Genua-star) in pots containing peat substrate with increasing dose of nitrogen from (0.2 - 0.9) g.dm^3. They found that in all the cultivars, with increase in nitrogen dose, there was no difference in the number of branches. In another study, Nurzyńska-Wierdak (2011) studied the effect of nitrogen feeding and non-feeding on four basil cultivars (Kasia, Wala, Genua-star, and Opal) grown in a medium silty loam soil and found that foliar nitrogen application did not have significant any effect on the branching. Sifola and Barbieri (2006) reported no significant difference in morphological characteristics of basil cultivars with increase in the nitrogen fertilization. In this study for Treatment 1, no difference was seen in the branching and nodes between the basil plants grown using NST1 and PANST1 which is consistent with the literature.

For plant root length, basil plants grown using NST1 and PANST1 were significantly different with root length of basil plants grown using PANST1 being shorter. By visual observation it was noted that plant roots were white and healthy for NST1 and PANST1. Also, basil plants grown using PANST1 had less root hair as compared to plants grown on NST1 (Fig. 51). In hydroponic cultivation of plants, root development and growth are not as important as in soil cultivation. The main function of roots is to anchor the plants, and reach and absorb water. Root hair help to increase the surface area for water and nutrient absorption. Thus, in hydroponics, plant roots tend to be shorter due to easy availability of water and nutrients and they tend to have less root hair. Extensive root growth can prove to be detrimental to vegetative plant growth as roots eat up carbohydrates necessary for vegetative growth (Jones, 2014). In this study, basil plants grown using PANST1 had significantly shorter roots with less root hair as compared to plants grown on NST1. This could be because of more concentration of nitrates throughout the harvest period in the PANST1 which made the nitrogen uptake by the roots easier reflected by significantly shorter roots with less hair.

(a)   (b)
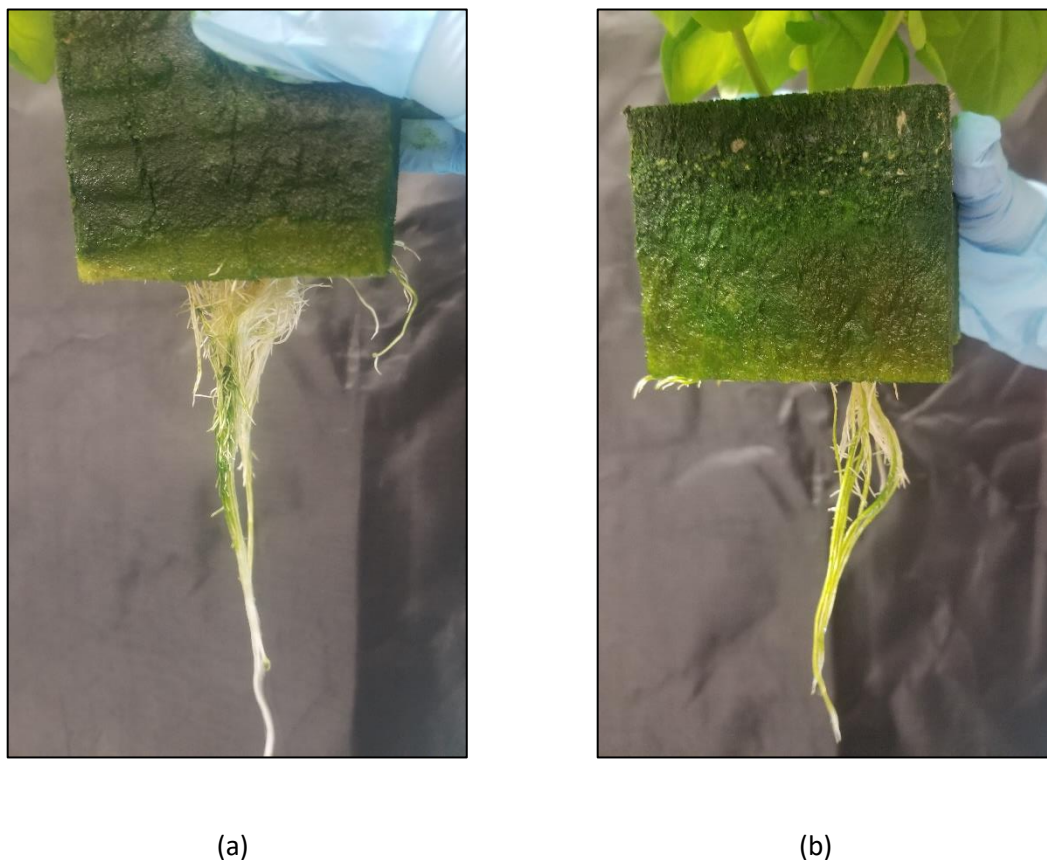
Figure 51: Roots of basil plants on day 21 for Treatment 1 (a) plants grown using NST1 (b) plants grown using PANST1

3.1.2.3 Leaf index

Leaf index was calculated after measuring the length and width of 10 basil leaves. This measurement was done on day 21 after harvesting the leaves from random plants from 2nd and 3rd nodes from top.

Table 17: Length, width, and leaf index of basil leaves for Treatment 1

Characteristic	NST1	PANST1
L (cm)	9.5 ± 0.7 [a]	10.0 ± 0.7 [a]
W (cm)	7.3 ± 0.8 [a]	7.5 ± 0.8 [a]
LI (cm^2) = 0.8 LW	56.1 ± 9.1 [a]	60.7 ± 8.5 [b]

(n = 30, data indicating different superscript letters across the columns in the same row are significantly different, one-way ANOVA, p<0.05)

No significant difference (p>0.05) was found in the length and width of basil leaves grown using NST1 and PANST1. But the leaf index was significantly more for basil leaves grown using PANST1 (Table 17). This increase in the LI can be attributed to the increased nitrogen content especially nitrate which is the most absorbed form of nitrogen by the plant roots throughout the growth period of basil plants. However, researchers have reported variable effect of increased nitrogen on basil leaf index.

According to Nurzyńska-Wierdak (2011), foliar application of nitrogen resulted in significantly more leaf length in Wala and Genua-Star basil cultivars whereas significant increase in leaf width was observed in Genua-Star and Opal. In another study Nurzyńska-Wierdak et al. (2012) reported significant decrease in leaf length and width of basil cultivars with increase in nitrogen dose from (0.2 - 0.9) g.dm^3. Sifola and Barbieri (2006) reported that the leaf areas of basil plants fertilized and not fertilized with nitrogen were not significantly different. The plants not fertilized produced less leaves to maintain the nitrogen content in the leaves. In hydroponic experiments done by Walter and Currey (2018) no significant difference was seen in the leaf

index with increase in the EC. However, according to Walch-Liu et al. (2000) nitrates are signaling molecules involved in leaf morphogenesis. After supplementing tobacco plants with nitrates, their leaf cytokinins (plant growth substances) levels significantly increased and significant leaf expansion was seen. Also, more available nitrate can lead to increased absorption of magnesium by the plant roots. This is due to the tendency of the plants to maintain the cation-anion balance. Magnesium is a key part of the chlorophyll structure and can enhance the photosynthetic process and thus can affect leaf expansion (Tränkner et al., 2018).

3.1.3 Colorimetric analysis of basil leaves

The colorimetric measurements were done on day 21 after harvesting 10 basil leaves from random plants from their 2nd and 3rd nodes. The L*, a*, b* color values were measured using Konica Minolta CR-410 colorimeter. The CIE scale color values (L*, a*, b*) directly relate to the eye-brain perception of the sample color. The L* value represents darkness and lightness of the sample. Higher the value, higher is the sample luminosity. The negative b* and positive b* values represent blueness and yellowness of the sample, respectively. The negative a* and positive a* values represent the greenness and redness of the sample, respectively (Pathare et al., 2013).

In this study, for Treatment 1, the a*, b* color values were significantly different ($p < 0.05$) for basil leaves grown using NST1 and PANST1 (Fig. 52). The L* value for basil leaves grown using NST1 was 40 ± 2, and PANST1 was 40.1 ± 2.1, and there was no significant difference. The a* value for leaves grown using NST1 was -17.2 ± 2.3 and for PANST1 was -19.1 ± 1.2 indicating that the basil grown on PANST1 were greener in color. The b* value for leaves

grown using NT1 was 19.7 ± 1.2 and for PANST1 was 15.7 ± 2.3 indicating that the basil leaves

grown using NST1 were yellower in color. According to Hu et al. (2010), there is a strong

correlation between L*, a*, b* color values and chlorophyll content and the color values can be

used to estimate the chlorophyll content in the plants. Thus, it can be implied that the basil

leaves grown on PANST1 had higher chlorophyll content.
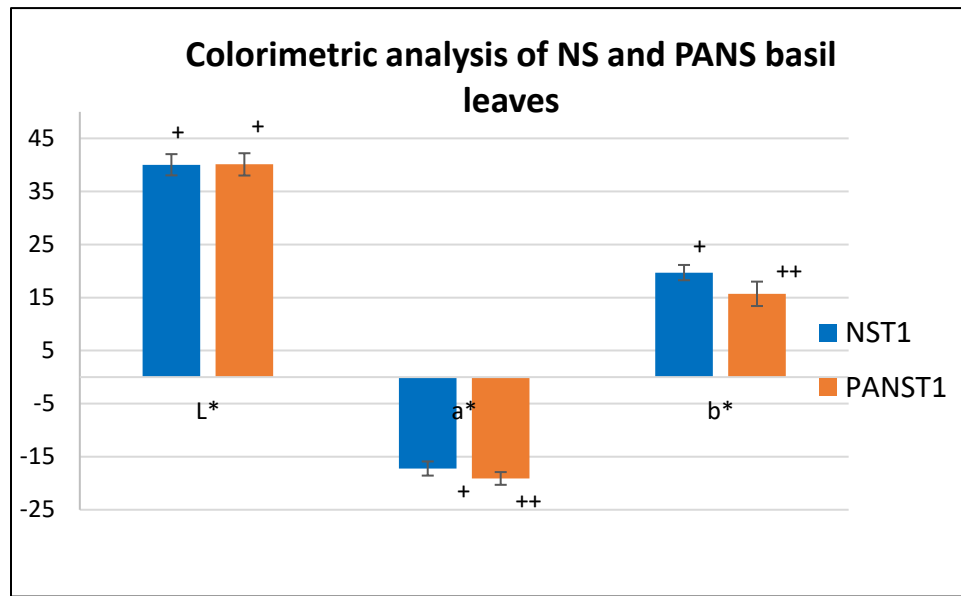
Figure 52: Colorimetric analysis of basil leaves for Treatment 1 to determine L* (lightness), a*

(green/redness), and b* (blue/yellowness) color values, (error bars indicate standard deviation,

n = 30, data not sharing the same number of symbols (+) are significantly different from each

other, one-way ANOVA, p<0.05).

To evaluate whether the above-mentioned difference in a*, b* values was significant to

cause visual difference in leaf color, the total color difference ΔE^*_{ab} was calculated using

Equation 2. This value represents if the color difference is detectable by eyes or not. ΔE^*_{ab} >3

indicates the compared sample colors were very distinct (Pathare et al., 2013). ΔE^*_{ab} value for

Treatment 1 was 4.1 ± 0.5 indicating the color of basil leaves grown using NST1 and PANST1 was very distinct.

Plant leaf color directly relates to the plant nutrition. Chlorophyll is one of the major pigments that establishes the plant leaf color (Hu et al., 2010). Nitrogen is a part of the chlorophyll structure. One of the first symptoms of nitrogen deficiency in plants is light green and yellow leaves (Wiedenhoeft, 2006). According to Agnihotri and Seth (2016), nitrate supplementation increased chlorophyll biosynthesis in tomato leaves due to up regulation of related genes. Also, more nitrate can lead to increased Mg uptake by the roots. Mg is a central part of the chlorophyll structure (Tränkner et al., 2018). Thus, it can be concluded that for Treatment 1, the color difference between basil leaves grown using NST1 and PANST1 could be due to more available nitrogen and nitrates to the basil plants grown on PANST1.

3.1.4 Texture analysis

The texture analysis was done on day 21 after harvesting 10 basil leaves from random plants from their 2nd and 3rd nodes. The analysis was done by performing a basil leaf puncture test (Fig. 53 a and Fig. 53 b) on Brookfield CT-3 texture analyzer.

(a)

Puncture test of basil leaves grown using PANST1
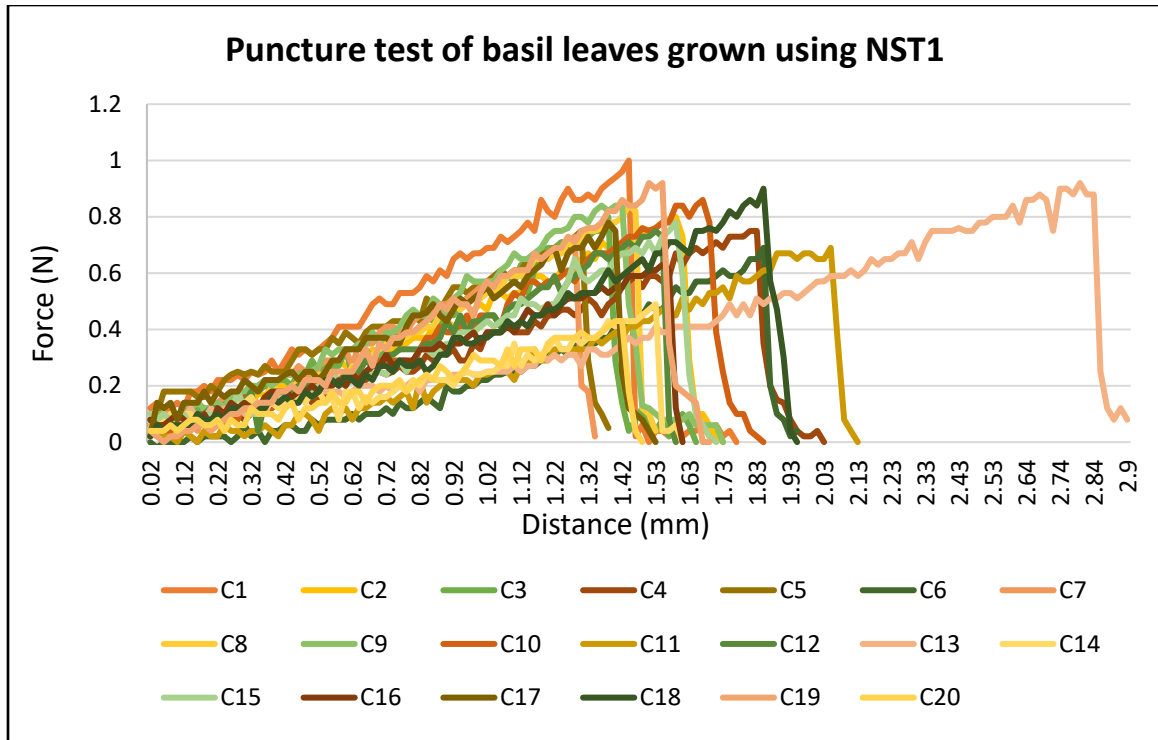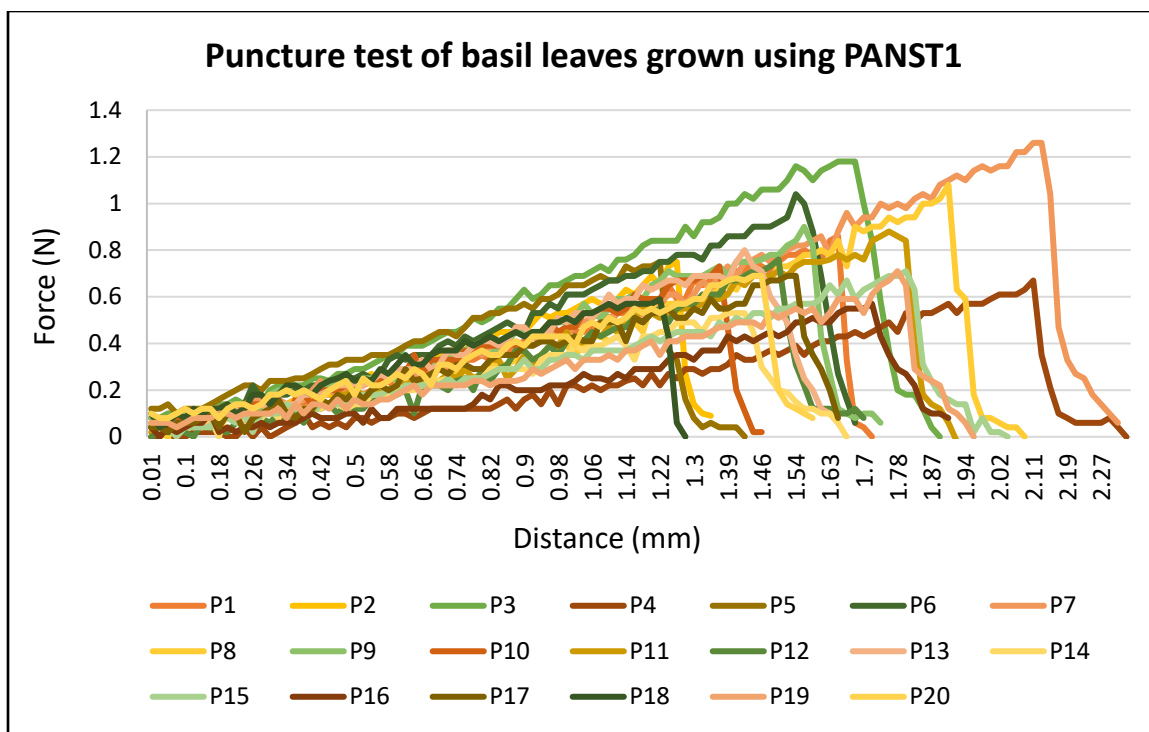
(b)

Figure 53: Force vs distance graph for Treatment 1 obtained during puncture test performed

using Brookfield CT-3 texture analyzer, 2 mm diameter pin probe, and a modified base plate (a)

puncture test of 20 basil leaves (C1 - C20) grown using NST1 (b) Puncture test of 20 basil leaves

(P1 – P20) grown using PANST1.

The puncture test (Fig. 53) was done to evaluate any difference in the peak rupture

force between basil leaves grown using NST1 and PANST1. The peak rupture force (Fig. 54)

between basil leaves grown using NST1 and PANST1 was not significantly different (p>0.05).

Young's modulus was calculated to investigate differences, if any, in the basil leaf elasticity. The

graph of puncture test (Fig. 53) was used to obtain the values for maximum deflection of the

basil leaf at the center and peak rupture force. These values were put in a formula (Eq. 3) to

obtain Young's modulus. Young's modulus values (Fig. 55) of basil leaves grown using NST1 and

PANST1 were not significantly different (p>0.05). The graph of force vs distance (Fig. 53)

obtained from the puncture test was also used to calculate the toughness of the basil leaves.

Toughness can be quantified using units of N.mm or mJ. The toughness of the leaves (Fig. 56)

was determined by calculating the area under the force vs distance graph (Gutiérrez-Rodríguez

et al., 2013) of the basil leaves. There was no significant difference (p>0.05) between toughness

basil leaves grown using NST1 and PANST1 (Fig. 55).
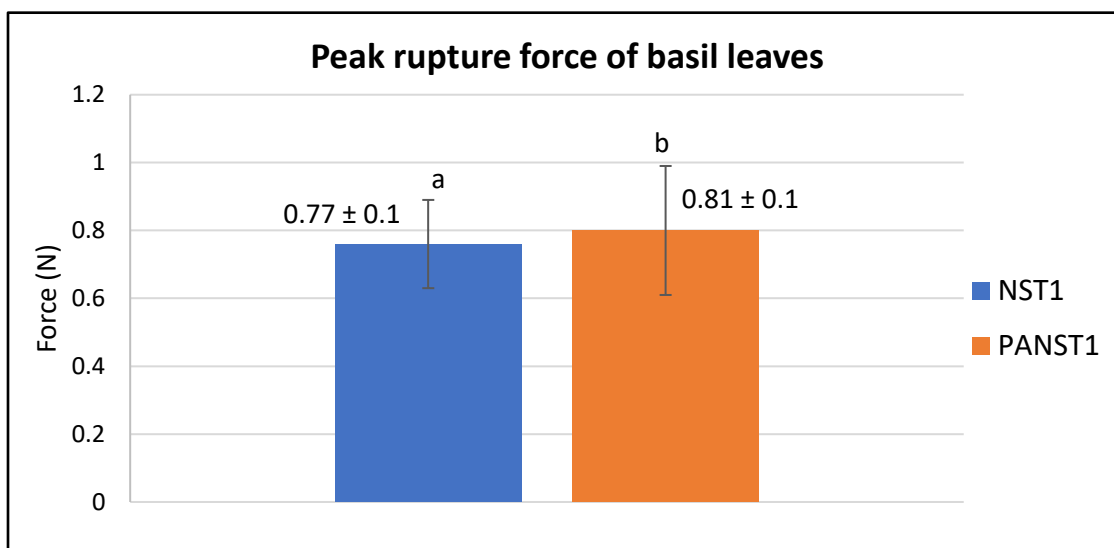
Figure 54: Peak rupture force of basil leaves for Treatment 1 (error bars indicate standard

deviation, n = 30, data not sharing the same letters are significantly different from each other,
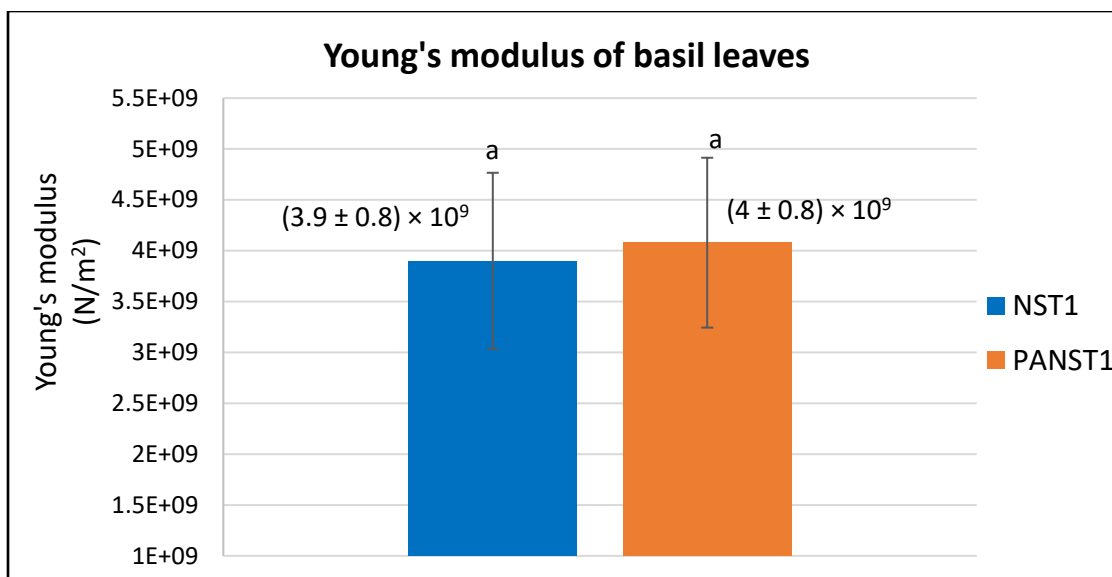
one-way ANOVA, p<0.05).

Figure 55: Young's modulus of basil leaves for Treatment 1 (error bars indicate standard deviation, n = 30, data not sharing the same letters are significantly different from each other, one-way ANOVA, p<0.05).
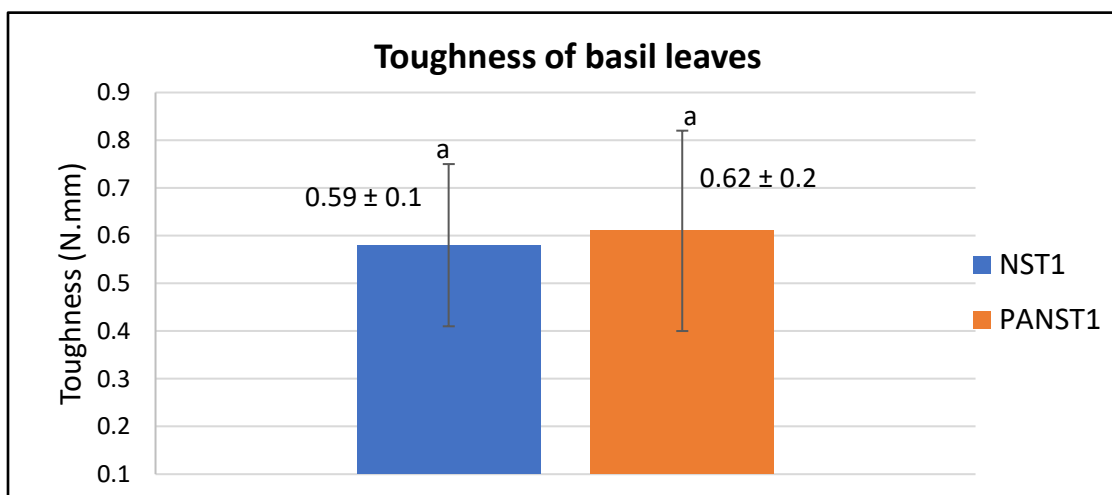
Figure 56: Toughness of basil leaves for Treatment 1 (error bars indicate standard deviation, n = 30, data not sharing the same letters are significantly different from each other, one-way ANOVA, p<0.05).

Previous research on effect of increased nitrogen on leaf texture indicates that increased nitrogen could lead to undesirable changes in the texture. Gutiérrez-Rodríguez et al. (2013) reported significant decrease in mechanical properties such as peak rupture force and toughness of hydroponically grown spinach leaves with increase in the total nitrogen of hydroponic solution from 50 ppm - 250 ppm. The spinach leaves became more fragile with increasing nitrogen. This was attributed to more plant growth resulting in increased intercellular air spaces. In this study for Treatment 1, even though basil plants grown using PANST1 had significantly more growth in terms of height and leaf index, there was no significant difference in the texture of the basil leaves grown using NST1 and PANST1 implying that there was no adverse effect of increased nitrogen and nitrate availability, and the presence of RONS on the texture of basil leaves.

3.1.5 Algae analysis

The algae concentration was measured at the end of every week using a Sedgewick cell and a microscope. The algae analysis of NST1 and PANST1 was done to investigate the effect of plasma (RONS) on algae growth in PANST1 and also to compare the algae growth between NST1 and PANST1. Visual inspection of NS and PANS flood tables was done frequently to investigate the algae build up (Fig. 58).

The algae concentrations of NS and PANS tanks were measured at the end of weeks 1, 2, and 3 (Fig. 57). The algae concentration in PANST1 was significantly less (p<0.05) than NST1 throughout the 3 weeks. At the end of week 1, PANST1 had 47.8 % less algae than NST1. At the

end of week 2, PANST1 had 50 % less algae than NST1. At the end of the harvest period (3 weeks), PANST1 had 22.2 % less algae than NST1.
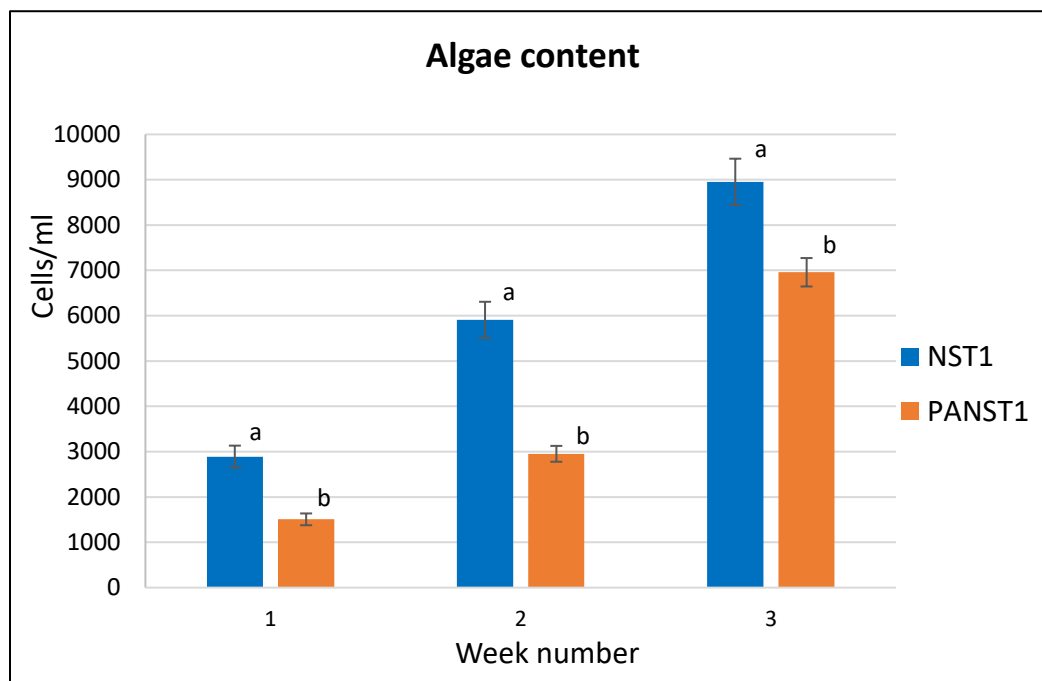
Figure 57: Algae concentration of NST1 and PANST1 at the end of every week for Treatment 1 (error bars indicate standard deviation, n = 9, data not sharing the same letters are significantly different from each other, one-way ANOVA, p<0.05).

According to the literature, plasma treatment can cause significant reduction in the algae. Dielectric barrier plasma treatment of microalgae suspension has been shown to cause severe damage to the algae cell morphology and result in reduced cell motility. This was attributed to cell oxidation reactions due to the presence of RONS and pH drop of the algae media (Tang et al., 2008). Another study used glow discharge cold plasma with air gas to get significant removal of microalgae in the surface water which was again attributed to oxidizing power of plasma species generated in the treated water (Kim et al., 2019). The significant reduction observed in the PANST1 algae concentration throughout 3 weeks (Fig. 57) could be

due to the plasma treatment involved in the generation of PANST1. The RONS species such as

NOx, N_2 could have helped in algae removal. In the first 2 weeks, the PANST1 had 47 % – 50 %

less algae than NST1. This reduction decreased at the end of week 3 to 22.2 % indicating that

with increasing time the inhibitory activity of the RNS species decreased significantly.

ORP is a measure to indicate oxidizing power of plasma treatment of the solution. The

ORP values of PANST1 were significantly more on day 1 and at the end of week 1 than NST1. By

the end of week 2 and week 3, there was no significant difference between the ORP values of

NST1 and PANST1. This was also reflected in difference of the rate of algae reduction in PANST1.

The higher ORP values for week 1 led to significant algae removal in PANST1. Even though the

difference in the ORP values at the end of week 2 was not significant, the PANST1 still had

approximately 50 % less algae than NST1 by the end of week 2. This could be due to 47 % less

algae at the end of week 1 in PANST1. As the oxidizing power of PANST1 further decreased in

week 3, the algae removal rate reduced to 22.2 % at the end of week 3.

(a)
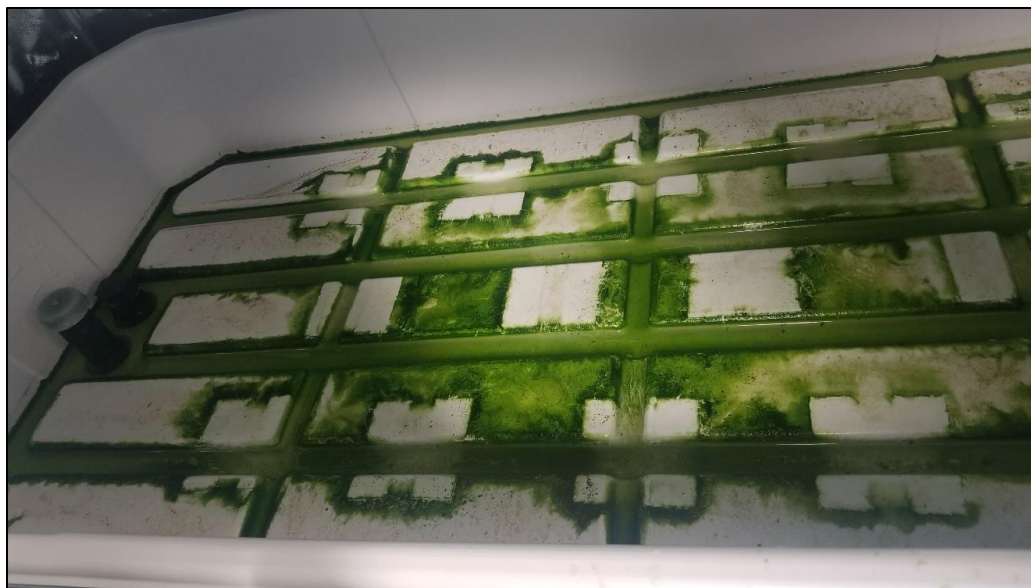
(b)

Figure 58: Flood tables for Treatment 1 at the end of 21 days after harvesting the basil plants (a) NS chamber flood table showing visibly more algae than (b), (b) PANS chamber flood table

Apart from the algae enumeration of NST1 and PANST1, flood tables were observed for the algae buildup. Fig. 57 (a and b) shows flood tables which were used to keep the basil plants inside NS and PANS chambers, respectively. During the harvest period, as the number of days increased more and more algae growth was seen in the NS flood table as compared to PANS flood table. By visual observation, at the end of the harvest period on day 21 more algae build up can be seen in the NS flood table (Fig. 58).

3.1.6 Microbial analysis

Microbial analysis was done using total plate count (TPC) test of basil leaves. This analysis was done to investigate differences if any in the microbial quality of basil grown using NST1 and PANST1. On day 21, total plate count test was performed and colony forming units (CFU) were noted down. The log CFU/g was estimated after counting the colonies from the first dilution. The total plate count for basil leaves grown using NST1 was 3.5 ± 0.06 log CFU/g and for basil leaves grown using PANST1 was 3.5 ± 0.07 log CFU/g and no significant difference ($p > 0.05$) was found between them. Low colony counts could be because of seemingly sterile conditions maintained in the NS and PANS chambers. Both the chambers were enclosed with limited exposure to outside environment. All the supplies needed for growing the seedlings such as trays were thoroughly cleaned. Before transferring the seedlings to NS and PANS chambers, the flood tables, lights, and inside of the chambers was thoroughly cleaned. Gloves were worn at all times when in contact with the seedlings. While handling the plants during the harvest period and during harvesting gloves were worn. The plant leaves were not exposed to any human contact or any outside material.

3.1.7 Fresh and dry weight analysis

Basil plants cut just above the surface of the grodan block were dried in a vacuum oven (Fig. 59) to obtain their dry weight and moisture content. Before placing the plants in the oven, their fresh weights were noted. Fresh weight and dry weight of basil plants was significantly different ($p < 0.05$) between basil plants grown using NST1 and PANST1. The basil plants grown using PANST1 had more fresh weight (15.4 % more) and dry weight (27.8 % more) than plants

grown on NST1. No significant difference (p>0.05) was found in their moisture content (Table 18).

Figure 59: Basil plants being dried in the vacuum oven at 40 °C on day 21 for Treatment 1

Table 18: Fresh weight, dry weight, and moisture content (w.b.) of basil plants for Treatment 1

Characteristic	NST1	PANST1
Fresh weight (g)	34.9 ± 1 [a]	40.3 ± 1.4 [b]
Dry weight (g)	3.0 ± 0.2 [a]	3.8 ± 0.2 [b]
Moisture (%)	91.4 ± 0.7 [a]	90.5 ± 0.8 [a]

(n = 9, data indicating different superscript letters across the columns in the same row are significantly different, one-way ANOVA, p<0.05).

It is very well researched that photosynthesis and nitrogen metabolism are dependent on each other. Nitrate reductase, nitrite reductase, glutamine synthetase are the main enzymes involved in nitrogen metabolism in the plants. Agnihotri and Seth (2016) found that nitrate supplementation significantly increased activities of these enzymes in tomato leaves. This led to increased photosynthesis and more plant growth. Nurzyńska-Wierdak et al. (2011) reported significant increase in the protein content with increased dose of nitrogen in basil, resulting in significantly more dry weight. Thus, for Treatment 1, the significant increase in the fresh and dry weight can be attributed to increased concentration of nitrates throughout the harvest period in the PANST1 (as indicated by its EC and nutrient analysis) which led to significantly more plant growth and might have increased the protein content.

3.1.8 Aroma analysis

Aroma analysis of the basil leaves was done to evaluate differences, if any, between the basil grown using NST1 and PANST1 in terms of their aroma profile. The samples dried using vacuum oven were further used for the aroma analysis. The analysis was performed using GCMS by Lara Brindisi in Dr. Jim Simon's Laboratory at Department of Plant Biology.

Table 19: Composition of basil leaves essential oil for Treatment 1

Aroma compound	NST1	PANST1
Linalool (%)	58.2 ± 7.4^a	69.5 ± 5^b
Eucalyptol (%)	12.1 ± 4.8^a	9.7 ± 2.8^b

Eugenol (%)	0.2 ± 0.3 [a]	0.5 ± 0.3 [a]
Methyl eugenol (%)	-	1.5 ± 0.5

(n = 6, data indicating different superscript letters across the columns in the same row are

significantly different, one-way ANOVA, $p<0.05$)

Based on their essential oil composition (Table 19), basil grown using NST1 and PAST1

both belonged to European chemotype which is rich in linalool. Basil leaves grown on PANST1

contained significantly more ($p<0.05$) linalool than basil leaves grown using NST1. Linalool is

responsible for imparting a sweet floral note to the bouquet of basil aroma. Methyl eugenol was

not detected in basil leaves grown using NST1, however, it was present in PANST1 basil leaves. It

is worth noticing that eugenol content of essential oil in basil leaves grown using PANST1 was

about double that of NST1. Based on the presence of methyl eugenol and more % of eugenol it

can be concluded that the basil leaves grown using PANST1 had an altered aroma profile and

that the slight, though significant increase in methyl eugenol could have be altered flavor or a

bit spicier note, which needs to be confirmed by sensory evaluation.

Increased concentration of linalool in basil leaves grown using PANST1 could be due

more available total nitrogen especially nitrates. Such increase was also reported by Zheljazcov

et al. (2008 a) and Nurzyńska-Wierdak et al. (2013). Zheljazcov et al. (2008 a) in their research

on nitrogen fertilization and its effect on basil essential oil profile found that apart from linalool

concentration, eucalyptol and eugenol too increased with nitrogen fertilization. However, in this

study, the eucalyptol concentration of PANST1 was lower than NST1, and even though the

eugenol % was more the difference was not statistically significant.

3.1.9 Tissue analysis of basil leaves

Basil leaves were harvested on day 21 and shipped to North Carolina State Department

of Agriculture (NCDA), Agronomic Division, Raleigh, NC for the tissue analysis. The tissue analysis

of the basil leaves can indicate what nutrients are being absorbed from NST1 and PANST1 by the

plants. The data from the tissue analysis could be compared with the sufficiency range of

nutrients. The sufficiency range indicates the optimum range for a nutrient in the specified crop,

below or above this range, the plant is said to be deficient or high, respectively, in that nutrient.

The sufficiency range of nutrients for basil (Bryson et al., 2014) is given in Table 20.

Table 20: Tissue analysis data of basil leaves for Treatment 1 along with sufficiency range for

basil

Nutrients	NST1	Sufficiency range	PANST1
N%	6.2 ± 0.2	4 − 6	6.4 ± 0.1
P%	0.8 ± 0.05	0.62 - 1	0.82
K%	6.8 ± 0.04	1.55 - 2.05	6.6 ± 0.2
Ca%	2.5 ± 0.07	1.25 - 2	2.5 ± 0.1
Mg%	0.5 ± 0.02	0.6 − 1	0.5 ± 0.02

S%	0.4 ± 0.04	0.2 – 0.6	0.45 ± 0.02
Mn (ppm)	38 ± 7.6	30 – 150	36.9 ± 5.8
Zn (ppm)	39 ± 4.95	30 – 70	38.8 ± 0.3
Cu (ppm)	11.6 ± 1.5	5 - 10	12.8 ± 0.8
B (ppm)	48.05 ± 1.3	25 - 60	54.3 ± 5.5
Fe (ppm)	109.5 ± 8.5	75 – 200	57.7 ± 56.2

From the Table 20, it can be seen that nutrients P, Ca, Mg, S, Mn, Zn, B, and Fe were within the sufficiency range for basil grown using NST1 and PANST1. For NST1 and PANST1, N, Cu, Ca were marginally above the sufficiency range indicating that both the samples were on the higher side for N, Cu, Ca content. The potassium level was very high in the basil tissue and Mg content in both basil samples was just below the sufficiency range. Thus, feeding plants with PANST1 could not be the reason for increased levels of N, Cu, Ca, and K, and marginally low level of Mg in the basil tissue.

The basil plants were observed almost every day and no visual signs of any deficiencies and toxicities were seen in the leaves, stems, and roots. The results for tissue analysis indicated excessive potassium in the basil tissue which can cause deficiencies of nutrients such as calcium, magnesium, manganese, and zinc (Rorabaugh et al., 2002). However, no such deficiencies were observed in the basil plants. The marginally less Mg in the basil tissue could be due to excess K. The increased level of N, Cu, Ca was not high enough to cause any toxicity symptoms to affect the plant growth. To understand the possibilities of why both basil samples contained more

tissue N, Cu, Ca, and high amount of K, it is important to look at the nutrient analysis data of NST1 and PANST1 given in section 3.1.10.

3.1.10 Nutrient analysis of NST1 and PANST1

Nutrient analysis was performed by shipping the NST1 and PANST1 samples to NCDA, Agronomic Division, Raleigh, NC. The samples were collected in plastic bottles on day 1 and day 21 for shipping. After the samples were shipped, it took approximately 7 days for the analysis to be completed. Nutrient analysis was done to see differences between the NST1 and PANST1 apart from their nitrogen content. Also, it was important to relate the visual symptoms of any deficiency or toxicity in the plants to the imbalances of the nutrient solution. Thus, along with the tissue analysis of the basil leaves, nutrient analysis of NST1 and PANST1 was performed.

To prepare PANST1, first, NS was prepared and then treated with gliding arc plasma. Hence, apart from the nitrogen content, the concentration of the rest of the nutrients should ideally be similar in NST1 and PANST1. From the Table 21 and Table 22, it can be seen that on day 1 apart from the total nitrogen and nitrate concentration, the concentrations for macronutrients such as P, Ca, Mg, S, and micronutrients such as Mn, Zn, Cu, B, Na, Cl, and Fe for NST1 and PANST1 were very close to each other. The increased level of K in the PANST1 could be due to the use of 10% potassium hydroxide to adjust the pH of PANST1. On day 21 (Table 21 and Table 22), increased concentrations of all the macro and micro nutrients as compared to day 1 are seen for both NST1 and PANST1. This could be due to water evaporation from the flood tables and the NS and PANS tanks and also due to relatively more absorption of water than the nutrients by plant roots (Resh, 1995). It can be observed that, even after 5-7 days (time

required to ship and analyze the samples) of the preparation of PANST1 (Table 21, day 1), the

total nitrogen and nitrates concentration is higher than NST1. For samples collected on day 21,

the PANST1 still had higher concentrations of total nitrogen and nitrates than NST1 (Table 21,

day 21).

Table 21: Macronutrient analysis of NST1 and PANST1 on days 1 and 21

	Total N (ppm)	Ammonium (ppm)	Nitrate (ppm)	P (ppm)	K (ppm)	Ca (ppm)	Mg (ppm)	S (ppm)
Optimum value	150-200	-	-	50	200	150	80	60
Day 1								
NST1	159 ± 2	9.4 ± 0.01	149.5 ± 1.5	49.1 ± 12.5	176 ± 38	142 ± 17	49.6 ± 4.9	77.4 ± 7.8
PANST1	170.5 ±2.5	9.9 ± 0.07	160.5 ±2.5	44.65 ± 6.8	191 ± 43	147.5 ± 21.5	51 ± 3.6	78.8 ± 7
Day 21								
NST1	239 ± 11	10.5 ± 3.4	228.5 ± 7.5	55.1 ± 2.7	187 ± 1	270 ± 7	64.7 ± 4	100.4 ± 2.5
PANST1	261 ± 17	8.8 ± 1.4	252 ± 18	67.4 ± 10	214 ± 9	312 ± 27	76.7 ± 4.1	121 ± 13

Table 22: Micronutrient analysis of NST1 and PANST1 on days 1 and 21

Day 1							
	Mn (ppm)	Zn (ppm)	Cu (ppm)	B (ppm)	Na (ppm)	Cl (ppm)	Fe (ppm)
Optimum range	0.8	0.3	0.2	0.7	-	-	2.8
NST1	0.38 ± 0.03	0.15 ± 0.01	0.17 ± 0.01	0.46 ± 0.04	26.3 ± 2.8	28.2 ± 14.8	2.4 ± 0.2
PANST1	0.4 ± 0.03	0.2 ± 0.03	0.2 ± 0.04	0.4 ± 0.04	26.4 ± 2.6	28.6 ± 2.6	2.5 ± 0.2
Day 21							
NST1	0.39 ± 0.1	0.34 ± 0.02	0.73 ± 0.03	0.57	40.5 ± 7.8	76.7 ± 10.8	2.1 ± 0.5
PANST1	0.32 ± 0.06	0.33 ± 0.09	0.54 ±0.16	0.68 ± 0.06	49.6 ± 0.1	98.1 ± 5.9	3.4 ± 1.7

The marginally low levels of Mg found in the tissue analysis of basil leaves could be due to less available Mg in NST1 and PANST1 as seen in the Table 22. From the tissue analysis, it was

observed that N, Ca, and Cu were just above the sufficiency range. However, their concentration

in NST1 and PANST1 on day 1 was at the optimum level (Table 21). But on day 21, their

concentrations were much higher (Table 22). This could explain why the N, Ca, and Cu

concentration of basil tissue was above the sufficiency range. K concentration of NST1 and

PANST1 was too near the optimum value on day 1 (Table 21). But unlike N, Ca, and Cu, the K

level was near the optimum concentration on day 21 as well (Table 21). Thus, the excess of K in

the basil leaf tissue cannot be explained based on the nutrient analysis data of NST1 and

PANST1.

3.1.11 Record of temperature, relative humidity, CO_2 concentration in NS and PANS

chambers, and of temperature of NST1 and PANST1 over the harvest period

The temperature inside NS and PANS chamber was recorded every hour and the data

were downloaded from the sensor. The Fig. 60 shows temperature inside NS and PANS

chambers for each day with standard deviation.
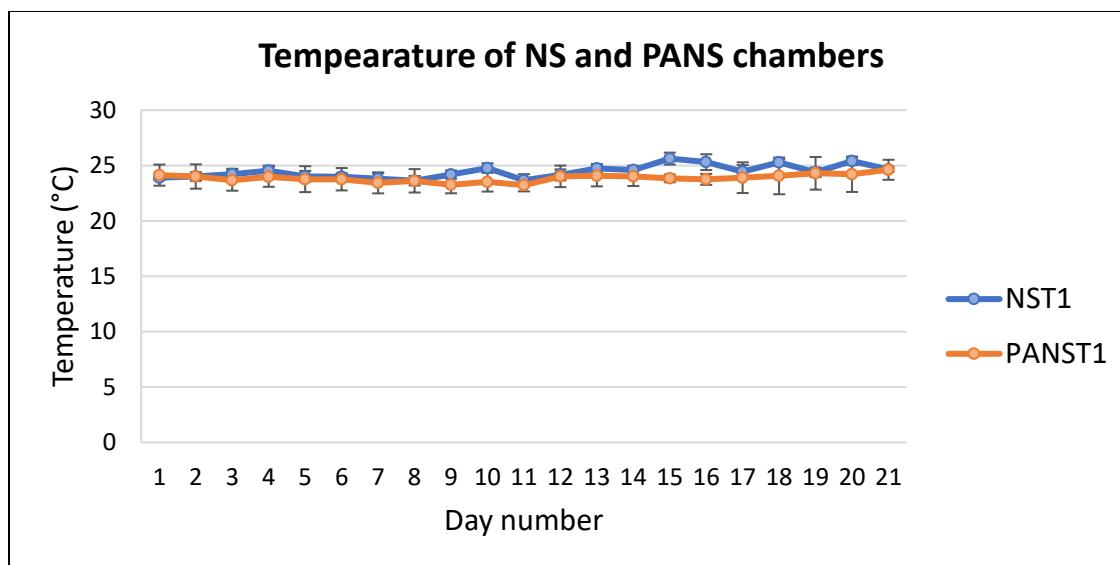
Figure 60: Average daily temperature of NS and PANS chambers for Treatment 1 over the

harvest period (error bars indicate standard deviation, n = 3).

As seen in the Fig. 60 the average temperatures on each day inside the both the

chambers were similar. The relative humidity inside both the chambers was recorded every 1 h

and the data were analyzed after downloading them from the sensor.
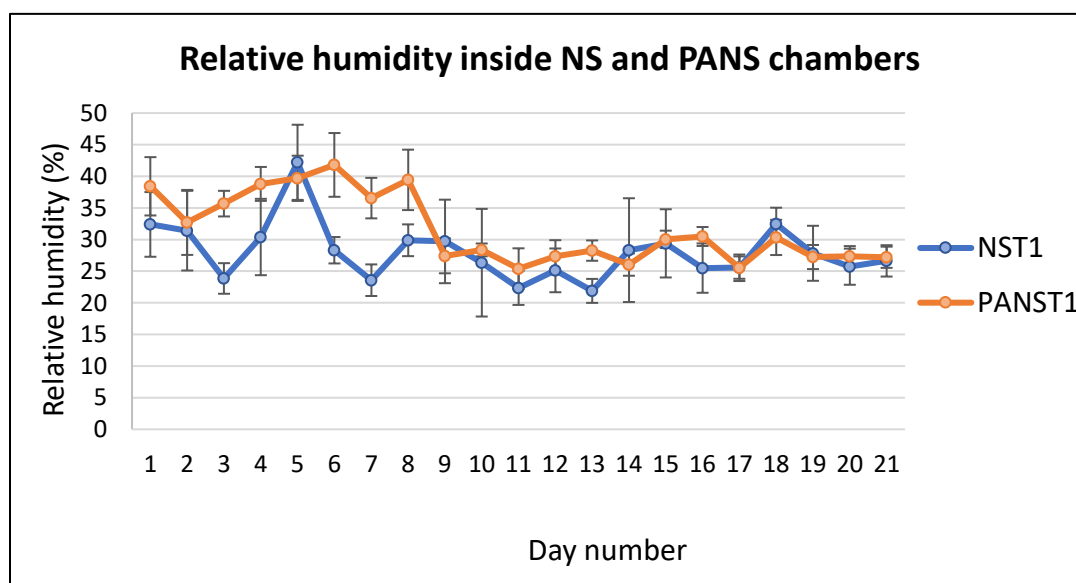
Figure 61: Average daily relative humidity of NS and PANS chambers for Treatment 1 over the harvest period (error bars indicate standard deviation, n=3).

As seen in the Fig. 61, there was a significant variation in the average relative humidity between the two chambers for first 8 days, after which both the chambers had similar levels. The initial variation in the average chamber relative humidity could be due to improper ventilation in the chambers. The CO_2 concentration was too recorded every 1 h and was analyzed at the end of the harvest period. As seen in the Fig. 62, the average CO_2 concentration each day inside both the chambers followed similar trend throughout the harvest period except for last 2 days.
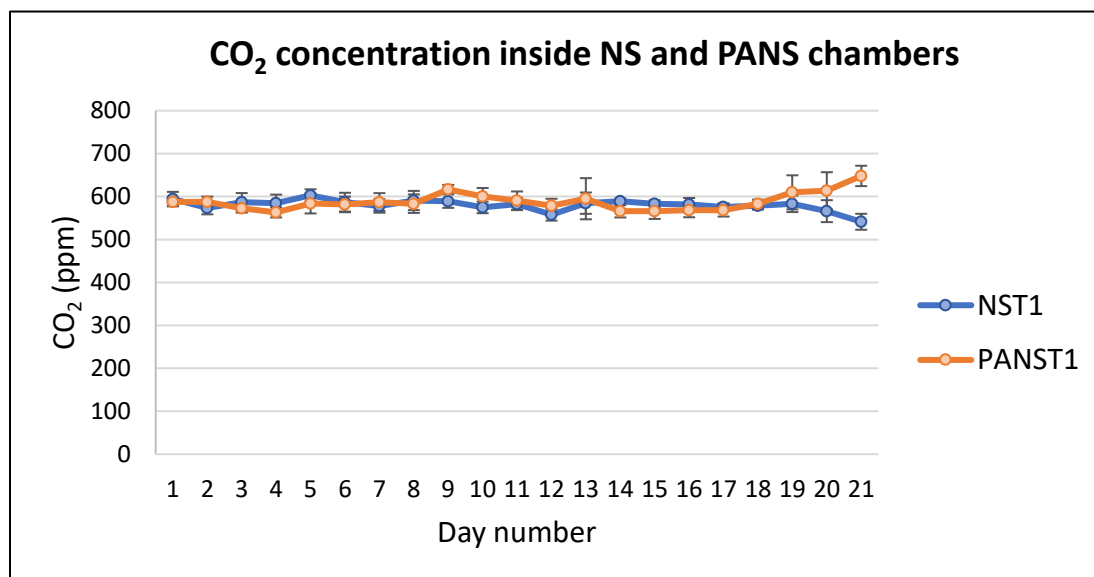
Figure 62: Average daily CO_2 concentration of NS and PANS chambers for Treatment 1 over the harvest period (error bars indicate standard deviation, n = 3).
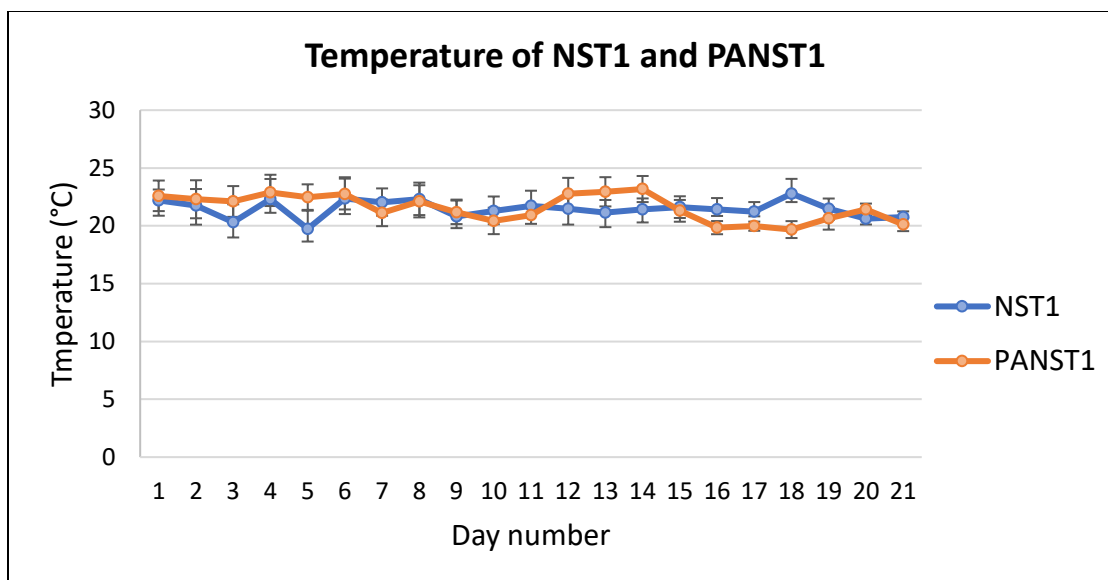
Figure 63: Average daily temperature of NST1 and PANST1 for Treatment 1 over the harvest period (error bars indicate standard deviation, n = 3).

The temperature of NST1 and PANST1 solutions was monitored every 1 h. The temperature of solution affects root zone temperature in hydroponic system. Ideally, the solution temperature should be close to chamber temperature to ensure proper nutrient absorption by basil plants for optimum growth.

The average daily temperatures of NST1 and PANST1 (Fig. 63) followed similar trend with some variation throughout the harvest period. As seen from Fig. 60 and Fig. 63, the average temperatures inside NS and PANS chambers and the average temperatures of NST1 and PANST1 solutions were close to each other throughout the harvest period. However, it could be noticed that the average temperatures NST1 and PANST1 (Fig. 63) were always (1-2) ^{0}C lower than the chamber temperatures (Fig. 60). This could be due to cold water used to prepare NST1 and PANST1 and also, the temperature of the room where the hydroponic system was run. The temperature of the room was set to approximately (18-20) ^{0}C using a thermostat.

The average chamber temperature for NS and PANS chambers was (23.4 ± 5.1) °C and (23.3 ± 0.5) °C, respectively, relative humidity for NS and PANS chambers was (34.5 ± 3.7) % and (33.7 ± 4.1) %, respectively, and CO_2 concentration for NS and PANS chambers were (575.1 ± 20) ppm and (580.3 ± 9.1) ppm, respectively. The temperature of NST1 and PANST1 was (21.2 ± 0.6) °C and (21.6 ± 1.2) °C, respectively.

3.1.12 Analysis of total energy consumption to grow basil plants for Treatment 1

The energy consumed by the lights, the pump, and the fan was measured using a wattmeter. The energy required to generate PANST1 was also measured using a wattmeter.

Table 23: The energy used in growing the plants for 21 days by PANS chamber for Treatment 1

Equipment	Time (h)	Energy (kWh)
Lights	336	72.2
Plasmatron	2.1	1
Pump	22.4	1.2
Fan	504	0.5
Total		74.9

The energy used by lights was calculated for a single day (16 h photoperiod for 21 days) and then was multiplied by the overall hours that the lights were kept on. Similarly, the

calculation was done for the fan which was kept on at all times for 21 days. The pump was run

for 1 h and then the reading obtained from the wattmeter was multiplied by the total time the

pump was run (8 min, 8 times a day for 21 days). The energy consumed by plasmatron was

measured for 1 run (approximately 2 h). After addition of all the consumed energy (Table 23),

the total energy consumed by the PANS chamber to grow 14 basil plants for 21 days was 74.9

kWh. To calculate the energy consumed by the NS chamber the plasmatron energy consumption

was subtracted from the PANS chamber energy to obtain the value of 73.9 kWh. Thus, it can be

concluded that use of plasma did not lead to any major increase in the energy consumption and

most of the energy was consumed by the lights.

3.1.13 Water consumption by the hydroponic system for Treatment 1

The NST1 and PANST1 solutions on day 1 in chamber tanks were 50 L each. Out of which

approximately 20 L each was left on day 21. Thus, the water consumed by each system was 30 L.

Out of this consumed water, plants only used the water that was present in them on day 21

which was calculated by multiplying their fresh weights with moisture content. Thus, the total

water used by plants in each chamber was obtained which was 2 % and 2.1 ± 0.2 %, respectively,

for NS and PANS chambers. The % water unused was approximately 40 % for both the chambers

and % water evaporated was approximately 58 % for both chambers. From these %, it can be

concluded that very little water was actually being used by the plants. And major % of the water

was evaporated from the flood tables and tanks. The energy that was needed for this water

evaporation was provided by the lights.

3.2 Treatment 2

In this section results for basil grown using multiple plasma treatments will be presented and discussed. The Treatment 2 (n=3) was done to evaluate the differences in the growth and the quality of basil plants after using fresh NS (NST2) and PANS (PANST2) on day 8 and day 15. In Treatment 1, the NST1 and PANST1 were prepared on day 1 and then the same solutions were used throughout the harvest period of 21 days without adjusting their EC values and nutrient content. Thus, as the days passed, the EC increased, and nutrient proportions changed.

For Treatment 2, the EC and nutrient proportions were brought to the optimum level by discarding the NST2 and PANST2 on day 8 and day 15, and using freshly prepared NST2 and PANST2 for week 2 and week 3. After discarding the solutions tanks were cleaned before adding fresh solutions to them. Same as that for Treatment 1, basil seedlings were grown using Rutgers Devotion DMR seeds and were transferred to NS and PANS chambers after 14 days (Figs. 64 a and 64 b). The seedlings were grown for 21 days inside the NS and PANS chambers and were harvested and analyzed for their growth and quality parameters on day 21 (Figs. 64 c and 64 d).

The average air temperature of NS and PANS chamber was (23.3 ± 5) °C and (23.4 ± 0.6) °C, respectively, relative humidity of NS and PANS chamber was (34.3 ± 3.8) % and (33.9 ± 4.3) %, respectively, and CO_2 concentration inside NS and PANS chambers were (577.3 ± 20) ppm and (581 ± 9.3) ppm, respectively. The average light intensity on top of the plants was approximately 250 µmol/m^{2}s in both the chambers.

(a)

(b)

(c)

(d)

Figure 64: Growth of basil plants during harvest period of 21 days for Treatment 2 (a) basil plants in NS chamber on day 1, (b) basil plants in PANS chamber on day 1, (c) basil plants in NS chamber on day 21, (d) basil plants in PANS chamber on day 21.

3.2.1 Characterization of NST2 and PANST2

3.2.1.1 pH, EC, and ORP of NST2 and PANST2

For Treatment 2, fresh NST2 and PANST2 was used to water the basil plants for week 2 and week 3. At the start of each week (days 1, 8, and 15) their pH was adjusted to 5.8 using 10% phosphoric acid solution and 10% potassium hydroxide solution (Table 24). The pH of NST2 and PANST2 was monitored everyday throughout the harvest period and adjusted to 5.8, if it crossed 6 (Fig. 65).

Table 24: pH of freshly prepared NST2 and PANST2 on days 1, 8, and 15 for Treatment 2

Day	pH	NST2	PANST2
1	Initial	6.8 ± 0.02	3.58 ± 0.01
	Adjusted	5.80	5.81
8	Initial	6.81 ± 0.01	3.6 ± 0.1
	Adjusted	5.80	5.80
15	Initial	6.82 ± 0.03	3.6 ± 0.1
	Adjusted	5.80	5.80

Apart from at the start of weeks 1, 2, and 3, on days 4, 7, 11, 18, and 20, the pH of NST2 and PANST2 had to be adjusted to 5.8 as it went above 6 (Fig. 64). At the end of the harvest period the pH of NST2 and PANST2 was 5.92 ± 0.02 and 5.92 ± 0.02, respectively.
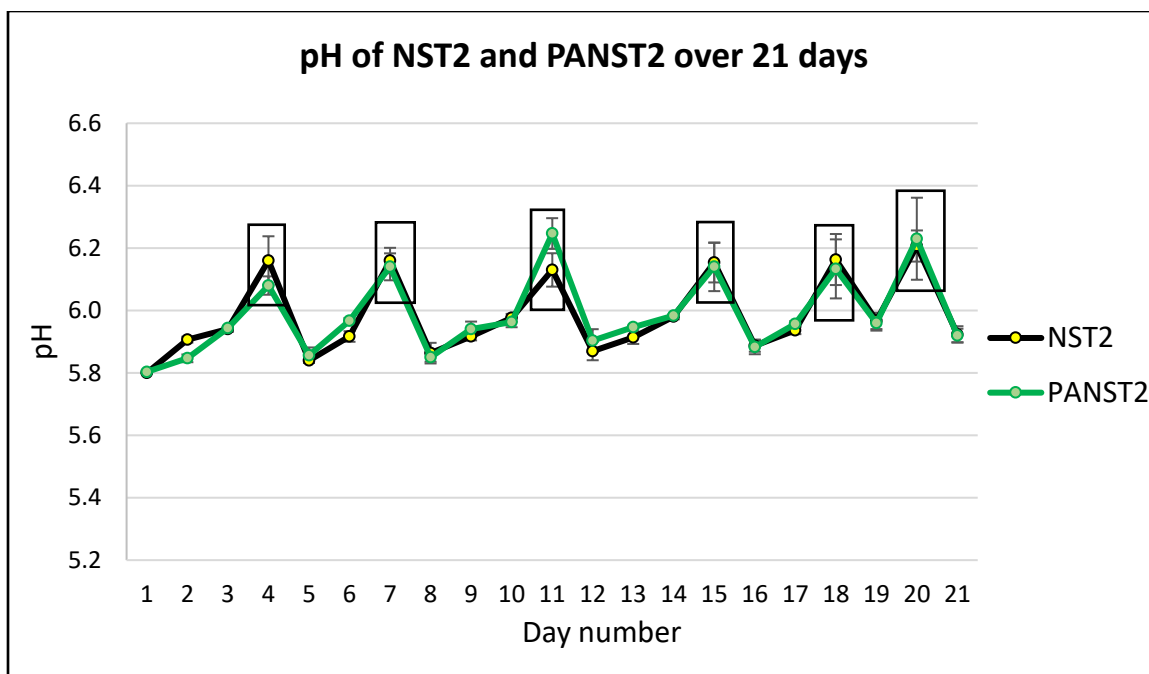
Figure 65: pH of NST2 and PANST2 for Treatment 2 over harvest period of 21 day and pH adjustment to approximately 5.8 on days 4, 7, 11, 15, 18 and 20, (error bars indicate standard deviation, n = 9).

Unlike the pH, EC was only monitored every day and not adjusted. On day 1 the EC of NST2 and PANST2 was (1.706 ± 0.01) mS/cm and 1.834 mS/cm, respectively. On days 8 and 15 (Table 25) the EC of NST2 and PANST2 was similar as that of that of day 1, as freshly prepared NST2 and PANST2 was used to water the basil plants for week 2 and week 3.

Table 25: EC (mS/cm) of fresh NST2 and PANST2 measured on days 1, 8, and 15 for Treatment 2

Day	NST2	PANST2
1	1.706 ± 0.01	1.834
8	1.756 ± 0.02	1.864 ± 0.02

15	1.747 ± 0.02	1.854

For weeks 1, 2, and 3, the EC for both NST2 and PANST2 increased with time (Fig. 66). This was due to evaporation of water from the flood tables and tanks, and more uptake of water as compared to nutrients by plant roots resulting in more concentration of nutrients in the water.
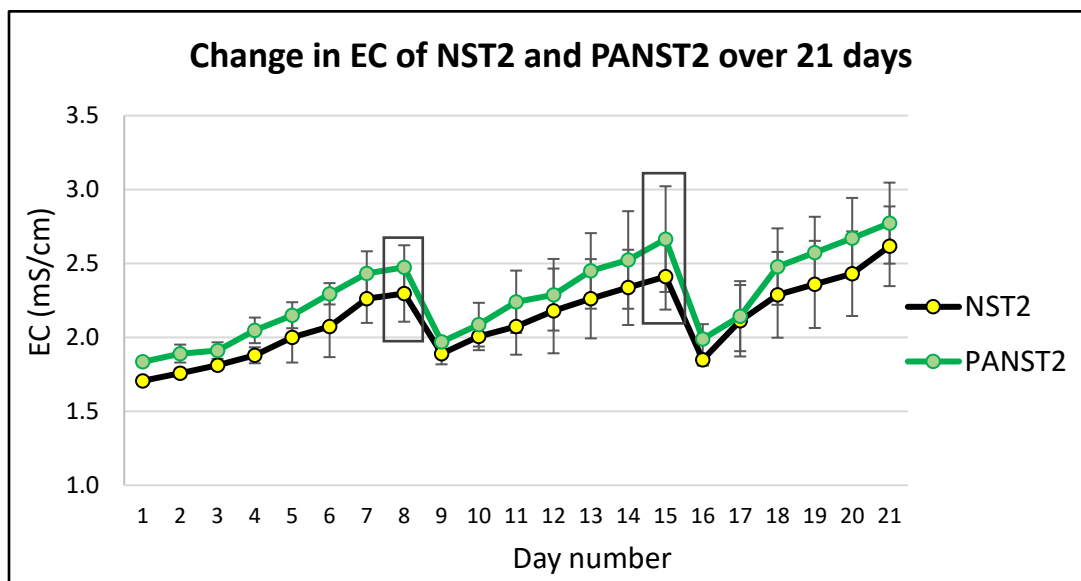
Figure 66: Change in the EC of NST2 and PANST2 for Treatment 2 over the harvest period of 21 days, (error bars indicate standard deviation, n = 9).

As seen in Fig. 66, there was a sudden drop in the EC of NST2 and PANST2 on day 9 and day 16. This drop was due to the use of fresh solutions for week 2 and week 3. It can be observed that the EC values for PANST2 over 3 weeks were always higher than NST2. This was attributed to the increased total nitrogen due to plasma treatment (Table 26). This was also

supported by the nutrient analysis data (discussed in section 3.2.10) for PANST2 which indicated

increased total nitrogen as compared to NST2 over the harvest period.

ORP value was measured as an indicator of the oxidizing power of PANST2 and to see

the difference in the ORP values of NST2 and PANST2 over time.
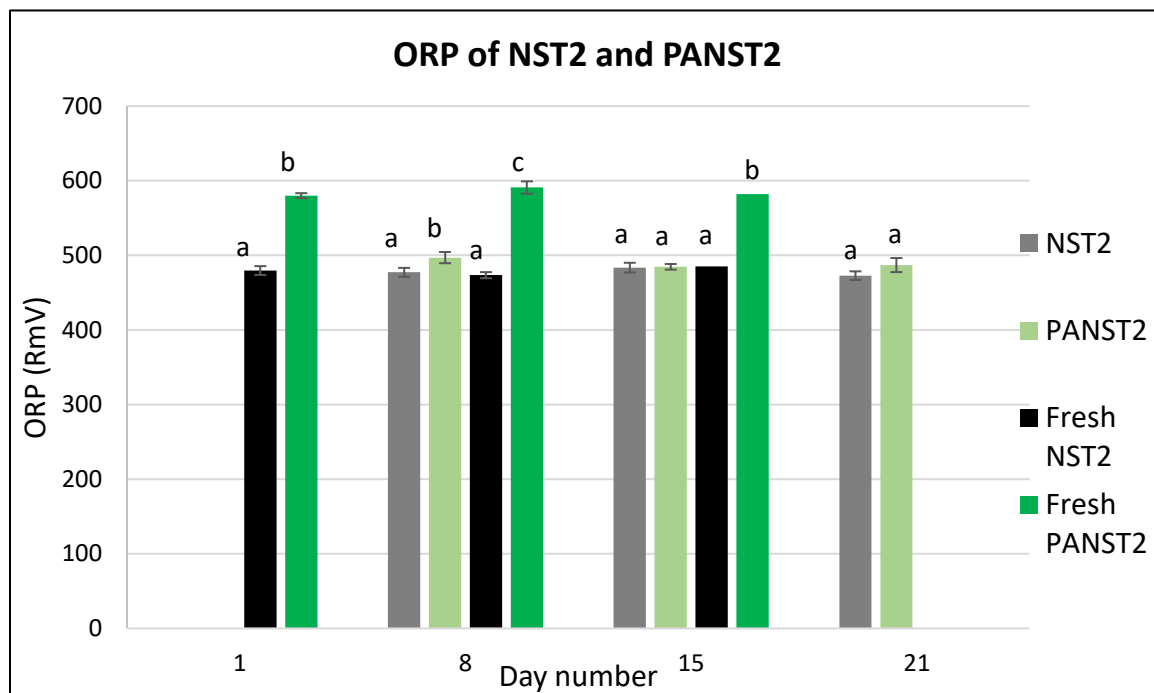
Figure 67: Change in the ORP of NST2 and PANST2 over the harvest period of 21 days, (error

bars indicate standard deviation, n = 9, data not sharing the same letter on the given day are

significantly different from each other, one-way ANOVA, p<0.05).

As seen in Fig. 67, the ORP values of fresh PANST2 at the start of weeks 1, 2, and 3 were

significantly higher (p<0.05) than NST2. At the end of week 1, on day 8, this difference was

reduced but was still significant. However, at the end of week 2 and week 3 there was no

significant difference (p>0.05) between the ORP value of NST2 and PANST2. This showed that

the oxidizing power of PANST2 was greatly reduced at the end of week 2 and week 3, even

though fresh PANST2 was used at the start of week 2 and week 3. This could be due to increased

presence of algae in NST2 and PANST2 which could have depleted the reactive species.

3.2.1.2 Nitrates, nitrites and hydrogen peroxide

For Treatment 2, the nitrates, nitrites, and hydrogen peroxide concentrations of freshly

prepared NST2 and PANST2 were measured on days 1, 8, and 15 (Table 26). After the plasma

treatment, the nitrate and nitrite concentrations increased by (38.84 ± 2.65) ppm and (9.54 ±

0.82) ppm on day 1 for PANST2, respectively, (38.5 ± 2.88) ppm and (9.58 ± 1.55) ppm on day 8

for PANST2, respectively, and (40.06 ± 1.46) ppm and (9.62 ± 1.28) ppm on day 15 for PANST2,

respectively. Hydrogen peroxide was not detected.

Table 26: Nitrates and nitrites concentration on days 1, 8, and 15 for Treatment 2 measured

using assays

Characteristic	Day 1		Day 8		Day 15	
	NST2	PANST2	NST2	PANST2	NST2	PANST2
Nitrates (ppm)	151 ± 1.24	189.8 ± 1.71	153.13 ± 2.44	191.63 ± 1.61	152.16 ± 1.31	192.22 ± 1.46
Nitrites (ppm)	9.04 ± 1.16	18.58 ± 0.94	9.12 ± 0.81	18.7 ± 1.13	8.73 ± 1.21	18.36 ± 0.8

Along with assay measurements, reactive species concentration was quantified using

test strips (Table 27). The data obtained using test strips measurement was consistent with data

obtained using assays. Increase of 40 ppm and 10 ppm in nitrates and nitrites, respectively, was

obtained after plasma treatment of nutrient solution.

Table 27: Nitrates and nitrite concentration on days 1, 8, and 15 for Treatment 2 measured using

test strips

Characteristic	Day 1		Day 8		Day 15	
	NST2	PANST2	NST2	PANST2	NST2	PANST2
Nitrates (ppm)	160	200	160	200	160	200
Nitrites (ppm)	10	20	10	20	10	20

3.2.2 Morphological analysis

3.2.2.1 Plant height analysis

For Treatment 2, all the conditions and parameters for seedling and basil plant growth

were same as that of for Treatment 1, only difference being use of fresh NST2 and PANST2 for

week 2 and week 3. The seedling heights on day 1 were (4.5 ± 0.12) cm and (4.45 ± 0.13) cm for

NST2 and PANST2, respectively. There was no significant difference ($p>0.05$) between the height

of seedlings on day 1. As seen in the Fig. 68, basil plants grown on PANST2 grew taller than NST2

after day 9. However, significant difference ($p<0.05$) between the plant heights was found only

on days 15, 18, and 21.
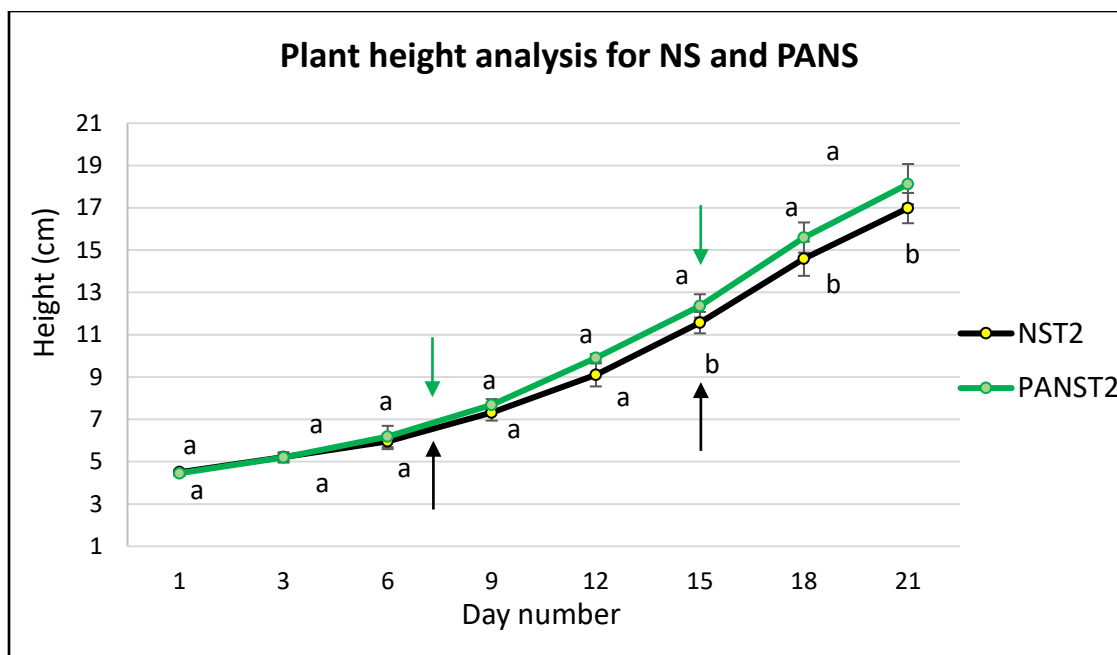
Figure 68: Plant height analysis of basil plants for Treatment 2 over the harvest period of 21 days, arrows indicating use of fresh NST2 and PANST2, (error bars indicate standard deviation, n = 42, data not sharing the same letter on the given day are significantly different from each other, one-way ANOVA, p<0.05). The arrows indicate the use of fresh NST2 and PANST2 for weeks 2 and 3.

The arrows in the Fig. 68 indicate the use of fresh NST2 and PANST2 for weeks 2 and 3. Even after using the fresh PANST2 for week 2 no significant difference was observed in plant heights until day 15. This could be due to the variable effect of nitrogen supplementation and increased EC on basil plant growth as discussed in section 3.1.2.1. On day 21 the basil plant heights were (16.9 ± 0.7) cm and (18.1 ± 0.9) cm for NST2 and PANST2, respectively, with basil plants grown on PANST2 being significantly more in height. The % increased height on days 15, 18, and 21 was comparable with Treatment 1. Thus, use of fresh solutions for week 2 and week 3 to water the plants did not result in any added benefit in terms of plant height.

3.2.2.2 Branches, nodes, and plant root length analysis

In this study, for Treatment 2, no significant difference (p>0.05) was found between basil plants grown using NST2 and PANST2 in terms of number of branches and nodes (Table 28). However, the root lengths of basil plants were significantly different (p<0.05) with plants grown using PANST2 having shorter roots with less root hair (Fig. 69). This could be due to the presence of more nitrogen in terms of nitrates leading to more availability of nitrogen to plant roots. As discussed in section 3.1.2.2, according to the previous research on basil growth and nitrogen supplementation, researchers did not find any morphological change in terms of number of branches and nodes with increase in the EC which was consistent with the data obtained from Treatment 2.

Table 28: Number of branches, nodes, and root length of basil plants grown using NST2 and PANST2

Characteristic	NST2	PANST2
Number of branches	7.9 ± 0.6^a	8.5 ± 0.3^a
Number of nodes	3.1 ± 0.1^a	3.2 ± 0.2^a
Root length (cm)	15.4 ± 0.8^a	13.9 ± 0.8^b

(n = 42, data indicating different superscript letters across the columns in the same row are significantly different, one-way ANOVA, p<0.05).

(a)   (b)

Figure 69: Roots of basil plants on day 21 for Treatment 2 (a) plants grown using NST2 (b) plants grown using PANST2

3.2.3.3 Leaf index

For Treatment 2, no significant difference was found between length and width of basil leaves. However, the leaf index of basil leaves grown using PANST2 was significantly more (Table 29).

Table 29: Length, width, and leaf index of basil leaves for Treatment 2

Characteristic	NST2	PANST2

L (cm)	9.34 ± 0.9 [a]	9.95 ± 0.71 [a]
W (cm)	7.86 ± 0.61 [a]	8.32 ± 0.5 [a]
LI (cm^2) = 0.8 LW	59.1 ± 9.67 [a]	66.52 ± 8.34 [b]

 (n = 30, data indicating different superscript letters across the columns in the same row

are significantly different, one-way ANOVA, p<0.05).

As discussed in section 3.1.2.3, the increase in leaf index can be attributed to increased

total nitrogen due to the presence of more nitrates in PANST2 over the harvest period. The

increased nitrogen could have led to its more uptake leading to increased leaf morphogenesis

resulting in more leaf index.

3.2.3 Colorimetric analysis

For Treatment 2, on day 21, L*, a*, and b* values of basil leaves harvested from 2nd and

3rd nodes were noted using Konica Minolta CR-410 colorimeter (Fig. 70). L* value for basil

leaves grown using NST2 was 38.2 ± 2 and for PANST2 it was 38.1 ± 1. No significant difference

(p>0.05) was observed in L* value indicating that there was no difference in terms of lightness

between the basil leaves. However, a*, and b* color values were significantly different. The a*

value of basil leaves grown using NST2 was -15.7 ± 5 and for PANST2 it was -21 ± 1.5. The more

negative a* value for PANST2 basil leaves indicates that the leaves were greener. The greener

color of basil leaves could be because of increased chlorophyll biosynthesis due to increased

nitrogen and nitrates in PANST2. The b* value for basil leaves grown using NST2 was 18.5 ± 2.7

and for it was PANST2 was 16.4 ± 1.6. Higher b* value for NST2 leaves indicated that the leaves were yellower.
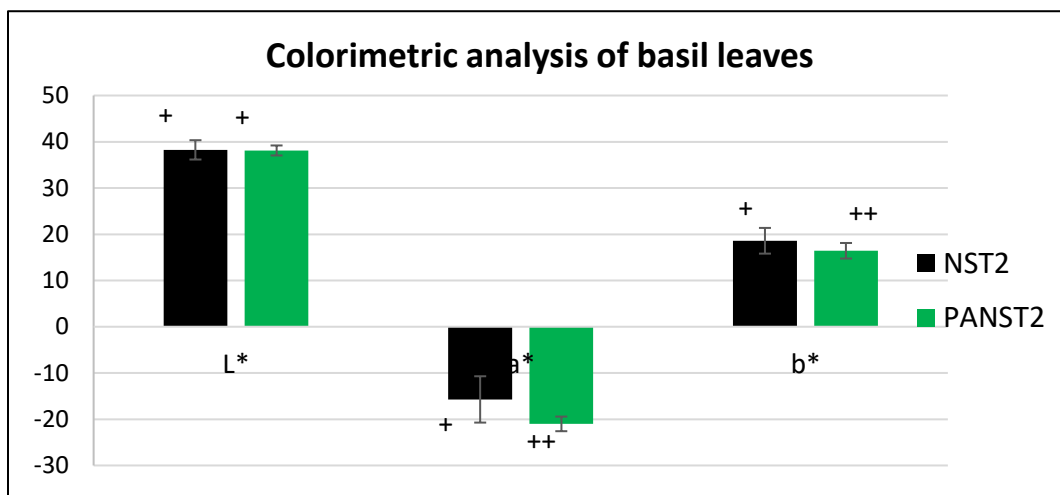
Figure 70: Colorimetric analysis of basil leaves for Treatment 2 to determine L* (lightness), a* (green/redness), and b* (blue/yellowness) color values, (error bars indicate standard deviation (n = 30, data not sharing the same number of symbols (+) are significantly different from each other, one-way ANOVA, p<0.05).

To evaluate the overall color difference between basil leaves grown using NST2 and PANST2, ΔE^*_{ab} value was calculated using Equation 2. ΔE^*_{ab} > 3 indicates significant color difference between the two samples distinguishable to human eyes. ΔE^*_{ab} for Treatment 2 was 5.7, indicating that there was visual color difference between the basil leaves.

3.2.4 Texture analysis

The texture analysis was done on day 21 to evaluate the differences in basil leaf texture in terms of rupture force, Young's modulus, and leaf toughness. The puncture test of the basil

leaves was done, as described in 2.2.8.6.1 to obtain a force vs distance graph (Fig. 71 a and b) to determine peak rupture force.

(a)

Puncture test of basil leaves grown using PANST2
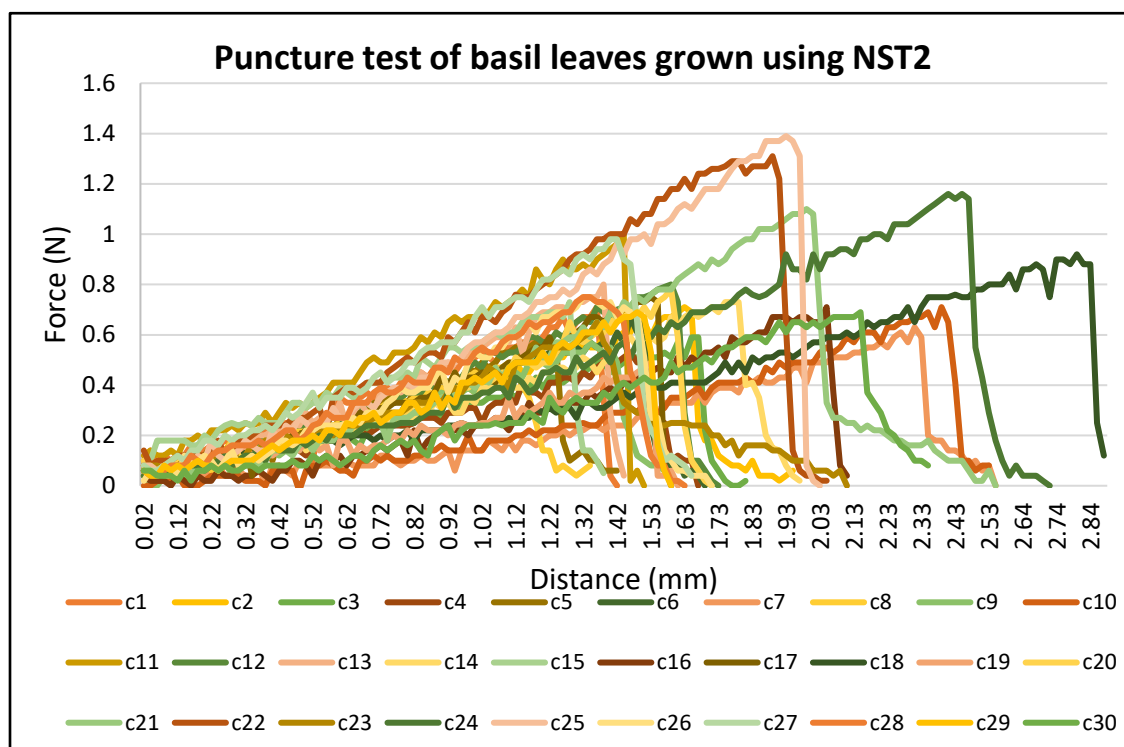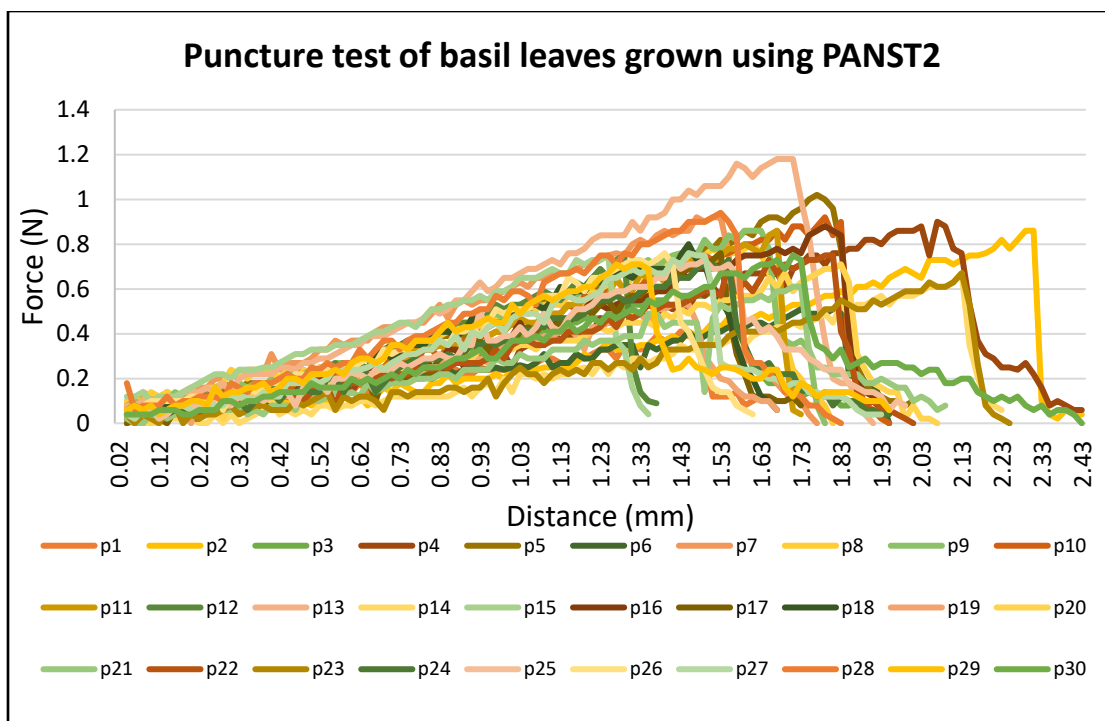
(b)

Figure 71: Force vs distance graph for Treatment 2 obtained by puncture test performed using

Brookfield CT-3 texture analyzer, 2 mm diameter pin probe, and modified base plate

(a) Puncture test of 30 basil leaves (c1 - c30) grown using NS

(b) Puncture test of 30 basil leaves (p1 – p30) grown using PANS

There was no significant difference ($p > 0.05$) in the peak rupture force of basil leaves

(Fig. 72). The force vs distance graph was further used to calculate area under the curve to

obtain toughness of the basil leaves (Fig. 73) and no significant difference ($p > 0.05$) was found in

the leaf toughness. Similarly, the Young's modulus (Fig. 74), which is a measure of substance

elasticity was too not significantly different ($p > 0.05$). This indicates that there no adverse effect

due to the use of PANST2 to water the basil plants on its leaf texture.
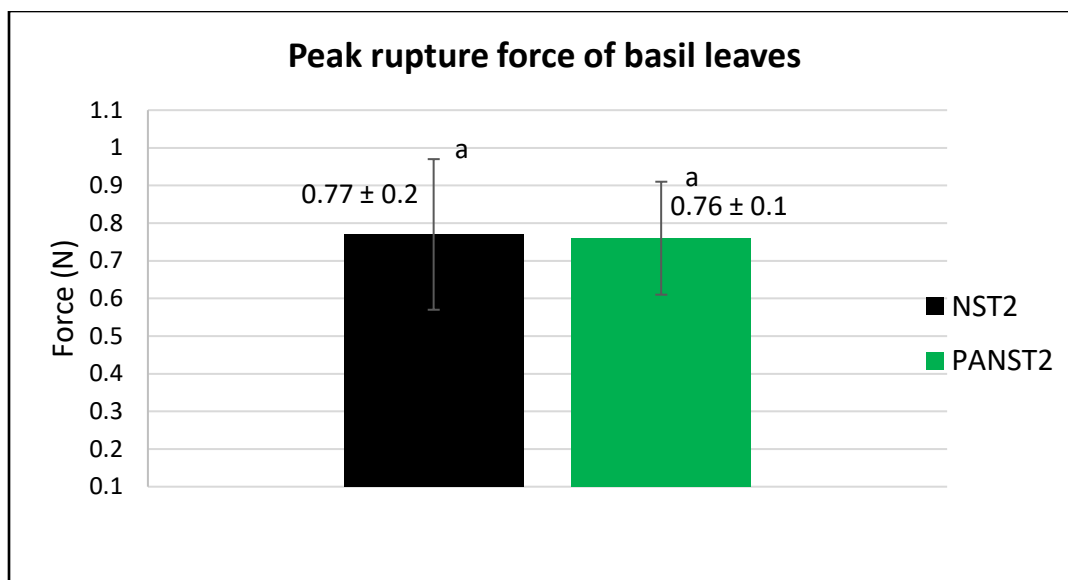
Figure 72: Peak rupture force of basil leaves for Treatment 2 (error bars indicate standard deviation, n = 30, data not sharing the same letters are significantly different from each other, one-way ANOVA, p<0.05).
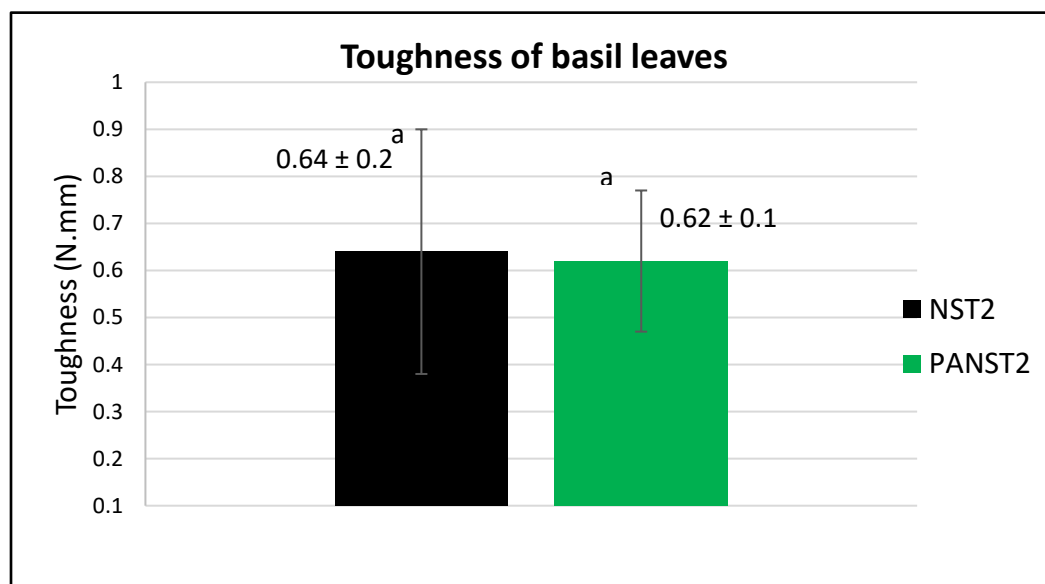
Figure 73: Toughness of basil leaves for Treatment 2 (error bars indicate standard deviation, n = 30, data not sharing the same letters are significantly different from each other, one-way ANOVA, p<0.05).
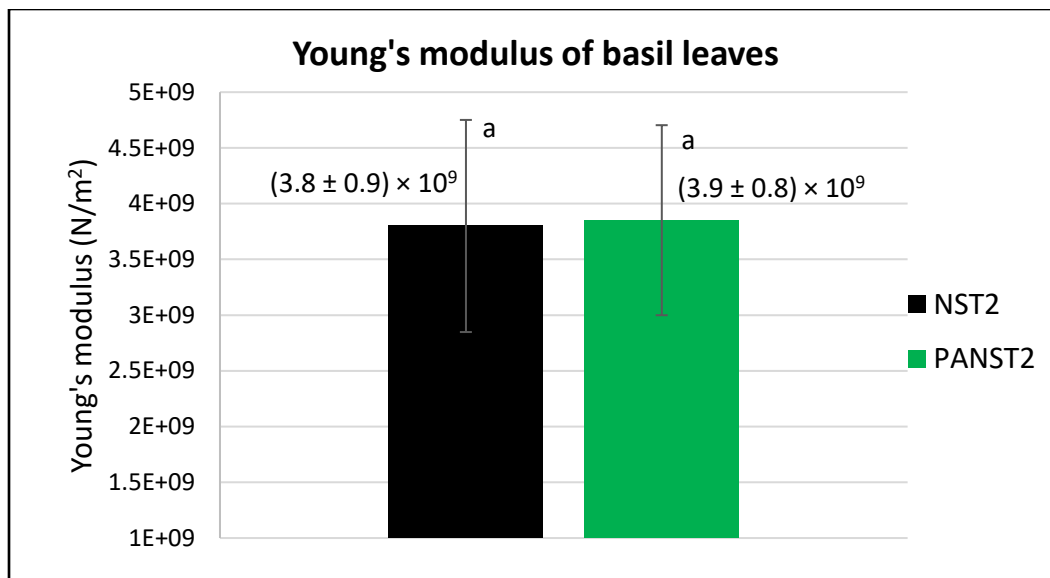
Figure 74: Young's modulus of basil leaves for Treatment 2 (error bars indicate standard deviation, n = 30, data not sharing the same letters are significantly different from each other, one-way ANOVA, p<0.05).

3.2.5 Algae analysis

Algae enumeration was done at the end of every week. On days 8 and 15 (end of week 1 and 2), the NST2 and PANST2 solutions were sampled before discarding to count the algae.
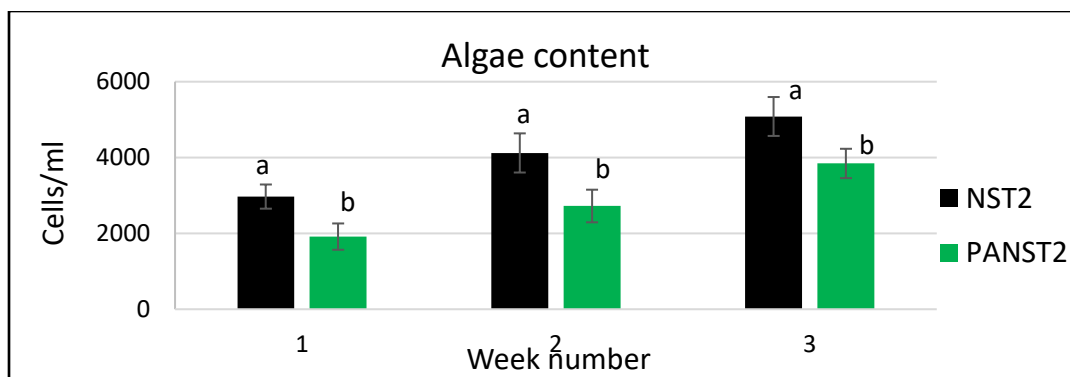
Figure 75: Algae concentration of NST2 and PANST2 in weeks 1, 2, and 3 for Treatment 2 (error

bars indicate standard deviation, n = 9, data not sharing the same letters are significantly

different from each other, one-way ANOVA, p<0.05).

As seen in Fig. 75, the algae concentration of PANST2 was significantly less (p<0.05) than

NST2 at the end of weeks 1, 2, and 3. At the end of week 2 and week 3, existing NST2 and

PANST2 from the chamber tanks were discarded and tanks were cleaned and again filled with

fresh NST2 and PANST2. However, the flood tables were not cleaned (because they had basil

plants growing in them) and still had visible algae growth. It can be observed from Fig. 75 that

the algae concentration for end of week 2 (4121.2 ±516 and 2722.4 ± 430) cells/ml for NST2 and

PANST2, respectively, and week 3 (5082.4 ± 513 and 3843.7 ± 387) cells/ml for NST2 and

PANST2, respectively, was more than that of for end of week 1 (2970.6 ± 319 and 1915.2 ± 347)

cells/ml for NST2 and PANST2, respectively, even though fresh solutions were used for week 2

and 3. This was due to the transfer of algae cells from the flood table to the NS and PANS

chamber tanks.

The reduction in the algae removal was consistent for first two weeks (35% and 33%)

but the reduction rate decreased (24%) at the end of week 3. The ORP value of PANST2 at the

start and end of week 1 was significantly higher than NST2 which could have caused the algae

removal. But even after using fresh PANST2 for week 2 and week 3, there was no difference the

ORP value when compared to NST2 at the end of week 2 and week 3. This could have caused the

reduction in the algae removal rate by the end of week 3.

(a)

(b)

Figure 76: Flood tables for Treatment 2 at the end of 21 days after harvesting the basil plants

(a) NS chamber flood table showing visibly more algae, (b) PANS chamber flood table

Apart from algae enumeration, the NS and PANS flood tables were observed frequently. Fig. 76 shows NS and PANS flood tables on day 21 after harvesting the basil plants. It can be seen that NS flood table had visibly more algae growth than PANS flood table. This can be attributed to use of fresh PANST2 and NST2 for week 2 and week3. The fresh NST2 solution did not have any algae cells which favored the growth of algae cells present in the NS flood table. Whereas the PANST2 solution had RONS which helped in algae removal from the PANS flood table.

3.2.6 Microbial analysis

To check any differences in the microbial quality of basil grown using NST2 and PANST2, total plate count (TPC) was done after harvesting the basil leaves on day 21. The total plate count of basil leaves grown using NST2 was 3.3 ± 0.1 log CFU/ g for PANST2 was 3.4 ± 0.1 log CFU/g and no significant difference ($p > 0.05$) was observed between them. The low colony counts can be attributed to seemingly sterile conditions maintained while growing the basil seedlings in the growth chamber and basil plants in the NS and PANS enclosed chambers. Gloves were worn while handling the basil seeds, during the transfer of basil seedlings to the NS and PANS chambers, and while harvesting the basil plants and leaves. The NS and PANS chambers were kept closed at all times. And care was taken while touching the plants.

3.2.7 Fresh and dry weight analysis

The fresh and dry weight of the basil plants were analyzed to see differences if any of using PANST2 as a growing solution for plant growth.

Table 30: Fresh weight, dry weight, and moisture content (w.b.) of basil plants for Treatment 2

Characteristic	NST2	PANST2
Fresh weight (g)	31.2 ± 3.5[a]	37.6 ± 2.4[b]
Dry weight (g)	2.6 ± 0.4[a]	3.4 ± 0.3[b]
Moisture (%) w.b.	91.4 ± 0.8[a]	90.7 ± 0.8[a]

(n = 9, data indicating different superscript letters across the columns in the same row are significantly different, one-way ANOVA, p<0.05)

From Table 30 it can be seen that the fresh and dry weight of basil plants grown using NST2 and PANST2 were significantly different (p<0.05). No significant difference was found between their moisture content. The basil plants grown using PANST2 had 20.5% and 30.7% more fresh and dry weight, respectively, compared to basil plants grown on NST2. This increase in the yield can be attributed to increased concentration of total nitrogen, in terms of nitrates in the PANST2 which could have led to more photosynthesis and increased protein content.

3.2.8 Aroma analysis

From Table 31 it can be seen that NST2 and PANST2 both were rich in linalool indicating that they belonged to European chemotype. Based on the essential oil composition (Table 30) no significant differences were found in aroma compounds such as linalool, eucalyptol, eugenol, and methyl eugenol.

Table 31: Composition of basil leaves essential oil for Treatment 2

Aroma compound	NST2	PANST2
Linalool (%)	78.4 ± 3.8[a]	80 ± 2.3 [a]
Eucalyptol (%)	9.3 ± 2.1 [a]	8.7 ± 0.8 [a]
Eugenol (%)	0.1 ± 0.04 [a]	0.07 ± 0.02 [a]
Methyl eugenol (%)	0.5 ± 0.1 [a]	0.8 ± 0.2 [a]

(n = 9, data indicating different superscript letters across the columns in the same row are significantly different, one-way ANOVA, $p<0.05$)

Even though the linalool and methyl eugenol content were more in the essential oil profile of PANST2, it was not significant. Thus, more availability of total nitrogen to basil plants fed on PANST2 did not have any effect on its essential oil profile.

3.2.9 Tissue analysis

The tissue analysis of basil leaves was done after harvesting the leaves on day 21. The
purpose of which was to know and evaluate the differences in the tissue nutrient content of
basil due to use of PANST2 to water the plants. From Table 32, it can be seen that for basil
plants grown on NST2 and PANST2, N, P, S, Mn, Zn, B, and Fe were within the sufficiency range
and Ca, Cu content was marginally above the sufficiency range. Mg content in both the basil
samples was marginally below the sufficiency range. However, K content was very high. Thus,
the plasma treatment involved in generation of PANST2 cannot be the reason for the increased
levels of Ca, Cu and very high content of K in basil leaf tissue.

Table 32: Tissue analysis data for basil leaves for Treatment 2 along with sufficiency range for
basil

Nutrients	NST2	Sufficiency range	PANST2
N%	5.9 ± 0.5	4 – 6	6 ± 0.2
P%	0.6 ± 0.04	0.62 - 1	0.6 ± 0.02
K%	5.81 ± 0.3	1.55 - 2.05	5.7 ± 0.3
Ca%	2.5 ± 0.04	1.25 - 2	2.4 ± 0.1
Mg%	0.5 ± 0.02	0.6 – 1	0.5 ± 0.03
S%	0.4 ± 0.02	0.2 – 0.6	0.4 ± 0.04

Mn (ppm)	43.4 ± 0.8	30 – 150	39.4 ± 3.1
Zn (ppm)	37.4 ± 10	30 – 70	36.3 ± 14.2
Cu (ppm)	10.9 ± 2	5 - 10	11.6 ± 3.2
B (ppm)	40.8 ± 6	25 - 60	41.5 ± 3.2
Fe (ppm)	101.9 ± 6.6	75 – 200	95.4 ± 6.7

The basil plant stems, leaves, and roots were observed almost every day to see any signs of nutrient deficiency or toxicity. The plants appeared healthy with no signs of Mg deficiency such as brown tips in leaves and roots. The marginally less Mg in basil tissue could be a symptom of excess K. Excess K could also lead to deficiency of Ca, Mn, and Zn. However, these nutrients are within the sufficiency range and no symptoms of their deficiency were seen in the growing plants. The high level of Ca and Cu was not enough to cause any toxicity symptoms such as stunted roots and iron deficiency. To understand the possible reasons of increased levels of Ca, Cu, and K, and marginally low level of Mg, it is important to evaluate the nutrient content of NST2 and PANST2 given in section 3.2.10.

3.2.10 Nutrient analysis of NST2 and PANST2

Nutrient analysis of NST2 and PANST2 was done after collecting the solution samples at the start and end of every week. The samples were then shipped to North Carolina State Department of Agriculture (NCDA), Agronomic Division, Raleigh, NC for the analysis. Table 33

and Table 34 give the nutrient analysis data of macro and micronutrients for fresh and used

NST2 and PANST2 for every week. Ideally the nutrient data for fresh NST2 and PANST2 for days

1, 8, and 15 should be the similar except for their total nitrogen and nitrate concentrations,

because NS was treated with gliding arc plasma to generate PANST2.

Table 33: Macronutrient analysis of fresh NST2 and PANST2 on days 1, 8, and 15

	Total N (ppm)	Ammonium (ppm)	Nitrate (ppm)	P (ppm)	K (ppm)	Ca (ppm)	Mg (ppm)	S (ppm)
Optimum value	150-200	-	-	50	200	150	80	60
Day 1								
NST2	153 ± 1	9.46 ± 0.2	143 ± 4.7	35.7 ± 1.7	142 ± 2.1	163 ± 4.9	46.8 ± 1	69.5 ± 3.7
PANST2	178.6 ± 2	11.4 ± 0.1	167.3 ± 1.6	36.4 ± 1.9	147.6 ± 3.2	194.6 ± 7.3	48.2 ± 0.7	71.5 ± 4.5
Day 8								
NST2	143 ± 6.6	9.14 ± 0.3	134 ± 4.3	31.4 ± 3.2	128.6 ± 15.3	152.6 ± 2.8	41.3 ± 4.6	62.2 ± 3.6
PANST2	163 ± 11	10.3 ± 0.6	147.5 ± 6.3	31.9 ± 5.2	134.6 ± 19.5	176.3 ± 10.5	45.5 ± 6.3	62.2 ± 7.6

Day 15								
NST2	148 ± 0.8	9.3 ± 0.1	138 ± 0.9	31.7 ± 2.9	133 ± 10.1	159.3 ± 2.4	42.4 ± 3.5	61.7 ± 4.5
PANST2	168.3 ± 7.7	10.5 ± 0.3	157.6 ± 7.5	33.1 ± 3.3	139 ± 14.1	181 ± 5.6	44.5 ± 4.6	64.6 ± 5.2

Table 34: Micronutrient analysis of fresh NST2 and PANST2 on days 1, 8, and 15

	Mn (ppm)	Zn (ppm)	Cu (ppm)	B (ppm)	Na (ppm)	Cl (ppm)	Fe (ppm)
Optimum value	0.8	0.3	0.2	0.7	-	-	2.8
Day 1							
NST2	0.45	0.15	0.17	0.42	34.9 ± 4.8	61.2 ± 9.7	3.4 ± 0.1
PANST2	0.4	0.2	0.2	0.37	34.9 ± 4.6	59.6 ± 8.9	3.4 ± 0.3

Day 8							
NST2	0.37	0.14	0.17	0.39	40.5 ± 7.6	68.3 ± 10.7	3.3 ± 0.4
PANST2	0.32 ± 0.06	0.33 ± 0.09	0.54 ±0.16	0.68 ± 0.06	49.6 ± 0.1	98.1 ± 5.9	3.4 ± 1.7
Day 15							
NST2	0.37	0.14	0.18	0.38	50 ± 8.5	85.1 ± 15.4	2.7 ± 0.5
PANST2	0.39	0.15	0.19	0.41	45.6 ± 5	77.3 ± 8.9	3.1 ± 0.7

Table 35: Macronutrient analysis of NST2 and PANST2 at the end of weeks 1, 2, and 3

	Total N (ppm)	Ammonium (ppm)	Nitrate (ppm)	P (ppm)	K (ppm)	Ca (ppm)	Mg (ppm)	S (ppm)
Optimum value	150-200	-	-	50	200	150	80	60
Day 8								

NST2	183 ± 29.3	10.3 ± 1.7	113.2 ± 69.4	32 ± 6.8	142.6 ± 23.1	231.3 ± 31.7	46.8 ± 7.5	69.9 ± 11
PANST2	201.6 ± 18.6	11 ± 1.1	190.3 ± 18	33.3 ± 6.8	158 ± 14.9	229.6 ± 17.1	51.3 ± 5	76.4 ± 6.4
Day 15							
NST2	179.5 ± 14.5	7.6 ± 2.4	172 ± 14.8	25.1 ± 7.3	144.6 ± 14	206.6 ± 15.4	49.4 ± 4.1	79.6 ± 9.3
PANST2	190.3 ± 21.6	8.2 ± 2.9	183 ± 18.8	24.3 ± 8.6	146 ±20	219.6 ± 23.4	49.7 ± 5.1	78.4 ± 10.5
Day 21							
NST2	181.6 ± 25.4	6.2 ± 3	175.6 ± 22.8	20.4 ± 10	142 ± 20.8	218.3 ± 20.9	50.8 ± 5.7	77.9 ± 7.6
PANST2	188 .6 ± 16.6	5.7 ± 3.1	182.6 ± 15.5	18.4 ± 9.8	155 ± 13.4`	221 ± 20	53.5 ± 6.1	82.9 ± 9.6

Table 36: Micronutrient analysis of NST2 and PANST2 at the end of weeks 1, 2, and 3

	Mn (ppm)	Zn (ppm)	Cu (ppm)	B (ppm)	Na (ppm)	Cl (ppm)	Fe (ppm)

Optimum value	0.8	0.3	0.2	0.7	-	-	2.8
Day 8							
NST2	0.33	0.38	0.49 ± 0.1	0.41	49.8 ± 8.1	84.7 ± 16.4	1.5 ± 0.2
PANST2	0.33	0.25	0.54 ± 0.1	0.45	52.4 ± 10.8	89.9 ± 20.4	1.6 ± 0.1
Day 15							
NST2	0.15 ± 0.1	0.28 ± 0.1	0.45	0.46	66.3 ± 13.7	120.5 ± 29.4	1.6 ± 0.1
PANST2	0.22	0.28 ± 0.1	0.42	0.46	65.6 ± 11.5	117 ± 27.8	1.7 ± 0.2
Day 21							
NST2	0.2	0.39 ± 0.1	0.46	0.45	73.9 ± 12	97.3 ± 76.5	1.8 ± 0.4
PANST2	0.17	0.18	0.46 ± 0.1	0.48	74.5 ± 11.6	132. 1 ± 24.1	1.8 ± 0.4

Apart from the total nitrogen and nitrate content of fresh NST2 and PANST2 on days 1, 8, and 15, the concentration of macronutrients such as P, K, Ca, Mg, and S, and micronutrients such as Mn, Zn, Cu, B, Na, Cl, and Fe are close to each other (Tables 33 and 34). The fresh PANST2 had increased total nitrogen ($\approx$ 20 ppm) and nitrate ($\approx$ 20 ppm) than fresh NST2 on days 1, 8, and 15. On day 21 (Table 35), at the end of the harvest period PANST2 still had more total nitrogen and nitrate than NST2. This increased N concentration could have caused increased growth of basil plants. For end of weeks 1, 2, and 3, the macro and micronutrient concentrations (Tables 35 and 36) increased for both NST2 and PANST2 as compared to their concentrations at the start of the week (Tables 33 and 34). This was due to water evaporation from the flood tables and tanks, and also more uptake of water than nutrients by the plant roots. This increased concentration correlates to increase in the EC of NST2 and PANST2.

From the tissue analysis, it was seen that the basil leaf tissue had Ca, K, and Cu concentrations above the sufficiency range. Ca concentration of NST2 and PANST2 on days 1, 8, and 15 (Table 33) was more than the optimum range. This concentration further increased at the end of each week (Table 35). This could be the reason for its increased concentration in the basil tissue. However, increased K tissue levels cannot be explained based on the nutrient analysis data as its level is always below the optimum value for NST2 and PANST2 (Tables 33 and 35). The Cu concentration was near the optimum value on days 1, 8, and 15 for fresh NST2 and PANST2 (Table 34). But its concentration almost doubled at the end of every week in NST2 and PANST2 (Table 36). This increase in the Cu concentration was due to water evaporation in NST2 and PANST2. This could have led to more Cu in the basil leaf tissue. The marginally low Mg in the tissue of basil leaves could be due to its below optimum value throughout the harvest period (Table 33 and Table 35).

3.2.11 Record of NS and PANS chamber temperature, relative humidity, CO_2 concentration,

and temperature of NST2 and PANST2 over the harvest period
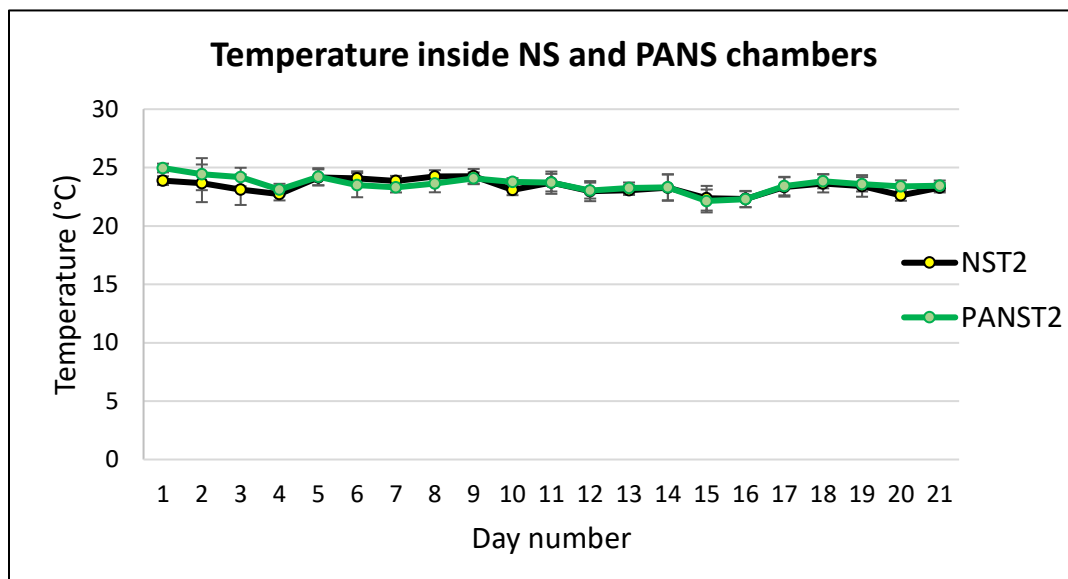
Figure 77: Average daily temperature of NS and PANS chambers for Treatment 2 over the

harvest period (error bars indicate standard deviation, n = 3).

Similar to Treatment 1, for Treatment 2, the temperature, the relative humidity, CO_2

inside the NS and PANS chambers, and the temperature of NST2 and PANST2 was monitored

every 1 h and was analyzed at the end of the harvest period. As seen in Fig. 77 the average

temperatures for each day follow the exact same trend with very little variation throughout the

harvest period. The average daily relative humidity (Fig. 78) for NS and PANS chambers was not

as stable as that of the average chamber temperatures but it too followed similar trend

between chambers throughout the harvest period.
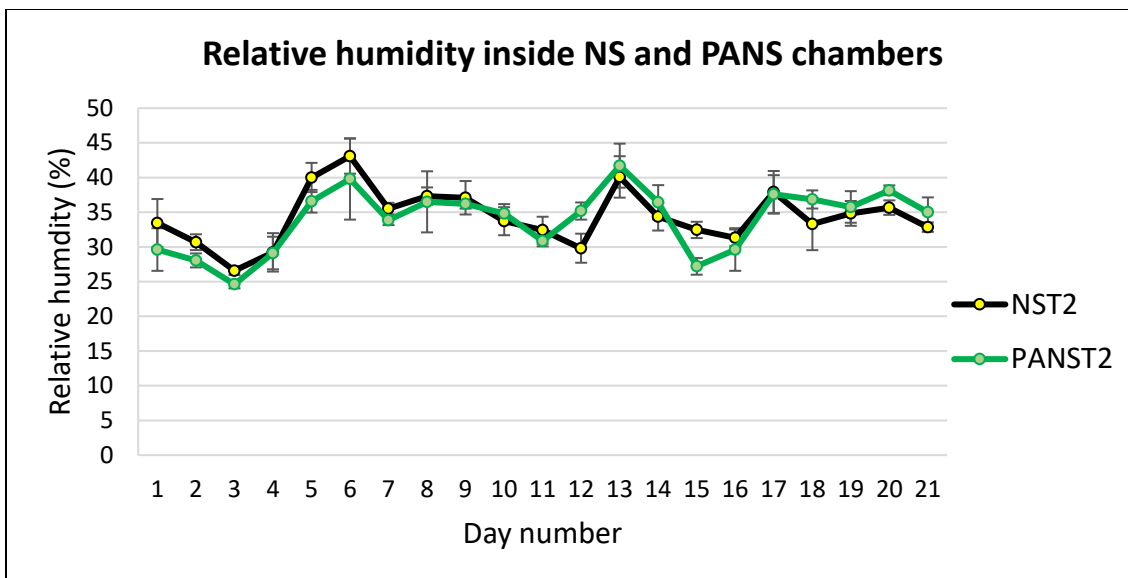
Figure 78: Average daily relative humidity in NS and PANS chambers for Treatment 2 over the harvest period (error bars indicate standard deviation, n = 3).
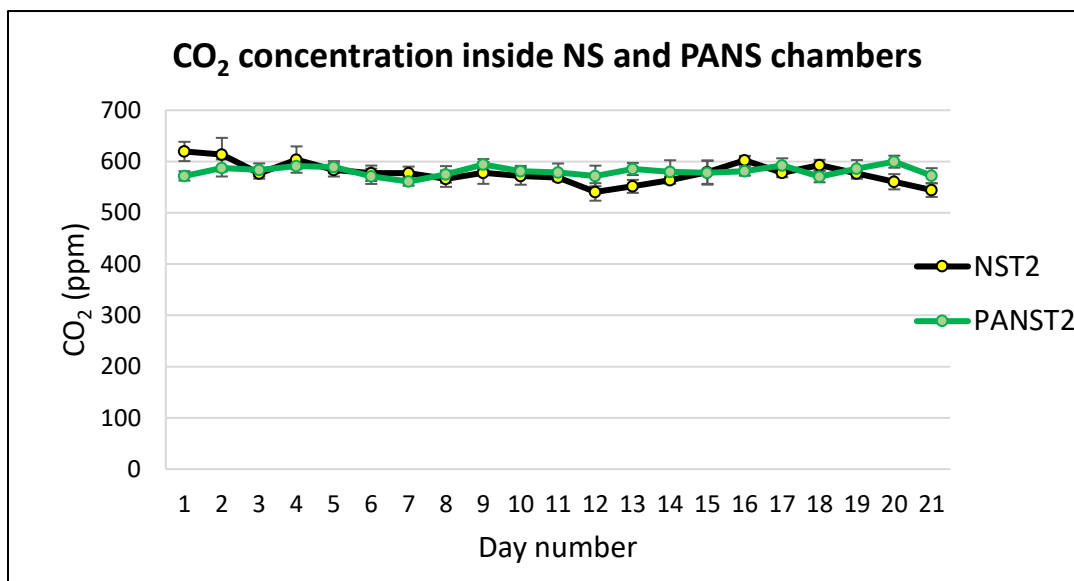
Figure 79: Average daily CO_2 concentration in NS and PANS chambers for Treatment 2 over the harvest period (error bars indicate standard deviation, n = 3).

The average daily CO_2 concentration inside both the chambers (Fig. 79) was close to each other with some variation in the first and last few days.
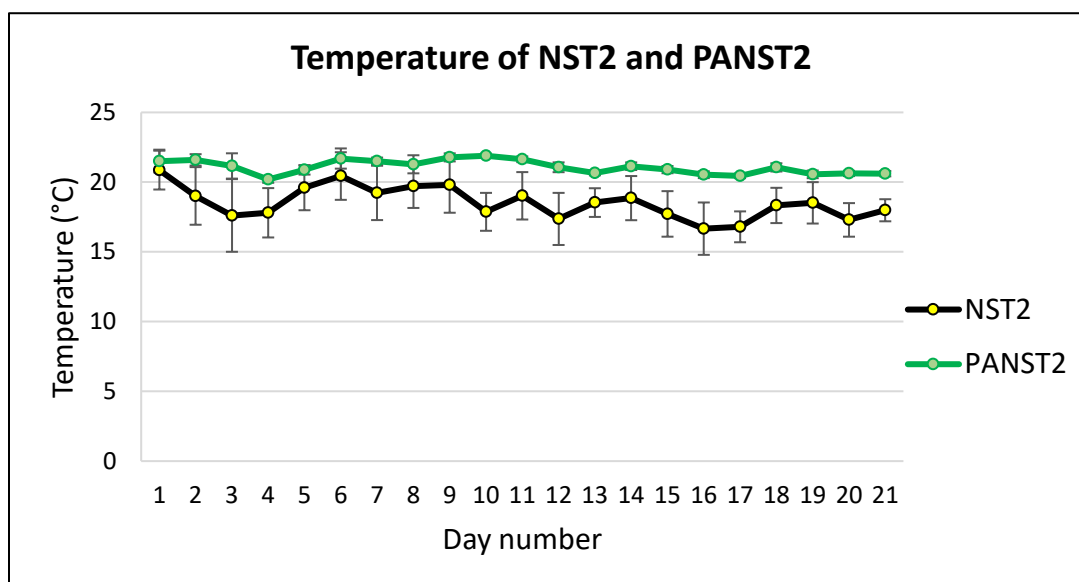
Figure 80: Average daily temperature of NST2 and PANST2 solutions for Treatment 2 over the harvest period (error bars indicate standard deviation, n = 3).

The average daily temperature of PANST2 as seen in the Fig. 80 was always approximately 2 °C higher than average daily temperature of NST2. This could be due to use of fresh solutions to water the plants for week 2 and week 3. Due to plasma treatment the temperature of PANST2 was higher at the start of every week and it always remained few degrees higher than NST2. This could also be due to the fact that Treatment 2 was done in the months of December to February resulting in colder water temperature of NST2. The temperature of PANST2 was exactly same as that of NST2 before the plasma treatment but it warmed up a little due to its plasma treatment.

The average air temperature of NS and PANS chamber was (23.3 ± 5) °C and (23.4 ± 0.6) °C, respectively, relative humidity of NS and PANS chamber was (34.3 ± 3.8) % and (33.9 ± 4.3)

%, respectively, and CO_2 concentration inside NS and PANS chambers were (577.3 ± 20) ppm and (581 ± 9.3) ppm, respectively. The temperature of NST2 and PANST2 was (18.5 ± 1.1) °C and (21 ± 0.4) °C, respectively.

3.2.12 Analysis of total energy consumption to grow basil plants

As explained in section 3.1.12 the total energy used by the system to grow basil plants over 21 days was calculated after taking the energy measurements with the help of a wattmeter. The only difference between energy used by the system in Treatment 1 and Treatment 2 was that the plasmatron was run 3 times instead of just at the beginning. The total energy consumed by PANS chamber was 76.9 kWh and NS chamber was 73.9 kWh. Similar to Treatment 1, the use of plasma did not cause any major change in the energy consumption (Table 37) and most of the energy required for plant growth was consumed by the lights.

Table 37: The energy used in growing the plants for 21 days by PANS chamber for Treatment 2

Equipment	Time (h)	Energy (kWh)
Lights	336	72.2
Plasmatron	6.3	3
Pump	22.4	1.2
Fan	504	0.5

Total		76.9

3.2.13 Water consumption by the hydroponic system for Treatment 2

Same as explained in section 3.1.13, the water consumption by the plants at the end of the harvest period was estimated. The water used by the plants was 1.9 % and 2 %, respectively, for basil plants grown using NST2 and PANST2, which indicated that very little amount was actually used for plant growth.

3.3 Comparison of use of PANS between Treatment 1 and Treatment 2

In this section, the data for PANST1 from Treatment 1 (section 3.1) and PANST2 from Treatment 2 (section 3.2) will be compared and discussed. This comparison will help to evaluate differences if any between single boost (PANST1) and multiple boost (PANST2) plasma treatments on basil plant growth and quality parameters. For PANST1 and PANST2 all the conditions and parameters for basil plant growth were same. Thus, changes, if any in the growth and quality of basil can be attributed to the differences in the use of PANS and its characteristics. For that it is necessary to first understand the differences in the EC and the nutrient composition between PANST1 and PANST2 over the harvest period.

3.3.1 Comparison of change in EC over the plant growth period
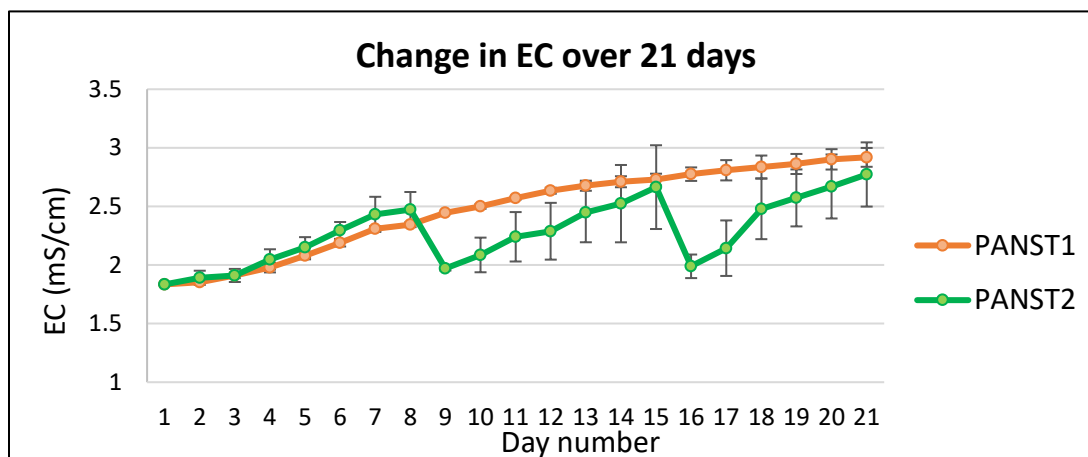
Figure 81: Change in the EC of PANST1 and PANST2 over the harvest period of 21 days, (error bars indicate standard deviation, n = 9).

The EC value of PANST1 and PANST2 for the first seven days was comparable to each other. However, the EC value of PANST1 was always higher than PANST2 after day 8. This could be due to more water evaporation from the flood tables and tanks. Another obvious reason for lower EC of PANST2 after day 8 was use of fresh PANS to water the plants for week 2 and week 3. Hence there was a sudden drop in the EC value of PANST2 as seen in Fig. 81 on days 9 and 16.

3.3.2 Comparison between nutrient analysis of PANST1 and PANST2

As seen in section 3.3.1, the EC of PANST1 was higher than PANST2 after day 8. This was also supported by the nutrient analysis data. It can be seen from the Table 38, that on day 1 the macro and micronutrient values between PANST1 and PANST2 were comparable except for Ca and K. However, on day 21 (Tables 38 and Table 39), macronutrients such as N, P, K, Ca, Mg, and

S, and micronutrients such as Mn, Zn, Cu, B, and Fe were considerably more in PANST1 than in PANST2. This was due to use of same PANST1 throughout the harvest period of 3 weeks which resulted in more water evaporation as compared to PANST2 as fresh PANST2 was used for week 2 and week 3.

Table 38: Macronutrient analysis of PANST1 and PANST2 on days 1 and 21

	Total N (ppm)	Ammonium (ppm)	Nitrate (ppm)	P (ppm)	K (ppm)	Ca (ppm)	Mg (ppm)	S (ppm)
Optimum value	150-200	-	-	50	200	150	80	60
Day 1								
PANST1	170.5 ±2.5	9.9 ± 0.07	160.5 ±2.5	44.65 ± 6.8	191 ± 43	147.5 ± 21.5	51 ± 3.6	78.8 ± 7
PANST2	178.6 ± 2	11.4 ± 0.1	167.3 ± 1.6	36.4 ± 1.9	147.6 ± 3.2	194.6 ± 7.3	48.2 ± 0.7	71.5 ± 4.5
Day 21								
PANST1	261 ± 17	8.8 ± 1.4	252 ± 18	67.4 ± 10	214 ± 9	312 ± 27	76.7 ± 4.1	121 ± 13

PANST2	201.6 ± 18.6	11 ± 1.1	190.3 ± 18	33.3 ± 6.8	158 ± 14.9	229.6 ± 17.1	51.3 ± 5	76.4 ± 6.4

Table 39: Micronutrient analysis of PANST1 and PANST2 on days 1 and 21

	Mn (ppm)	Zn (ppm)	Cu (ppm)	B (ppm)	Na (ppm)	Cl (ppm)	Fe (ppm)
Optimum range	0.8	0.3	0.2	0.7	-	-	2.8
Day 1							
PANST1	0.4 ± 0.03	0.2 ± 0.03	0.2 ± 0.04	0.4 ± 0.04	26.4 ± 2.6	28.6 ± 2.6	2.5 ± 0.2
PANST2	0.4	0.2	0.2	0.37	34.9 ± 4.6	59.6 ± 8.9	3.4 ± 0.3
Day 21							
PANST1	0.32 ± 0.06	0.33 ± 0.09	0.54 ±0.16	0.68 ± 0.06	49.6 ± 0.1	98.1 ± 5.9	3.4 ± 1.7
PANST2	0.17	0.18	0.46 ± 0.1	0.48	74.5 ± 11.6	132. 1 ± 24.1	1.8 ± 0.4

This difference in the macro and micronutrients between PANST1 and PANST2 over 3 weeks could have affected the basil plant growth and quality parameters. Increased concentration of total nitrogen and rest of the nutrients could lead to more plant growth and can affect the quality parameters.

3.3.3 Comparison of Morphological characteristics

3.3.3.1 Plant height analysis
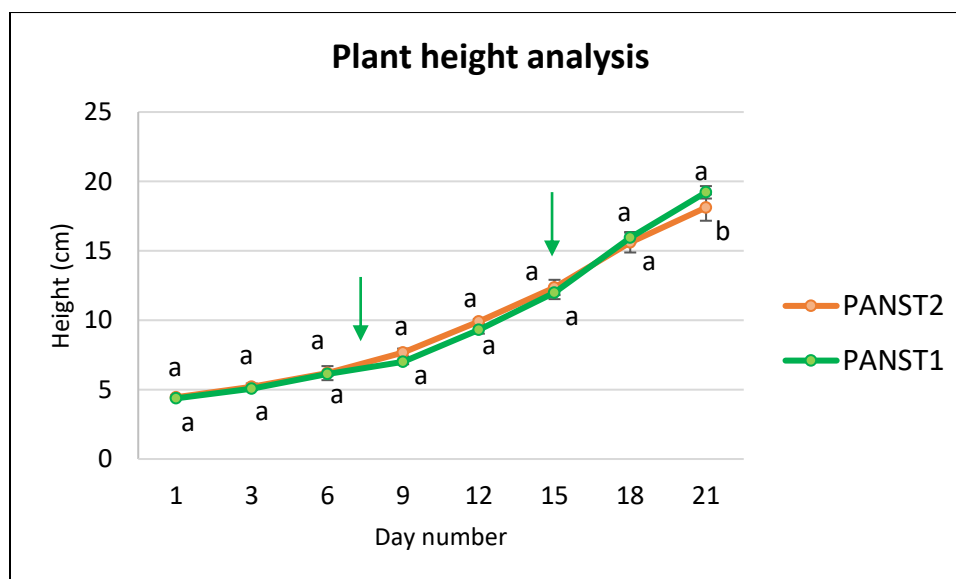
Figure 82: Plant height analysis for PANST1 and PANST2 over the harvest period of 21 days, arrows indicating use of fresh PANST2, (error bars indicate standard deviation, n = 42, data not sharing the same letter on the given day are significantly different from each other, one-way ANOVA, $p<0.05$). Arrows indicate use of fresh PANST2.

As seen in Fig. 82, basil plant height for PANST1 and PANST2 was not significantly different (p>0.05) on day 21. After day 15, as seen in the Fig. 82, PANST2 plants grew less tall than PANST1. This can be attributed to increased concentration of total nitrogen in PANST1 due to water evaporation and use of same PANST1 throughout the harvest period. As days passed, the EC of PANST1 was higher than PANST2 from day 8 to day 21 (Fig. 82). At the end of the harvest period on day 21, the PANST1 basil plants were significantly taller than PANST2. This could be due to more concentration of total nitrogen and nitrates in PANST1 from day 8 onwards.

3.3.3.2 Branches, nodes, and plant root length analysis

There was some morphological difference between basil plants grown using PANST1 and PANST2 (Table 40). Basil plants grown using PANST1 had significantly more (p<0.05) number of branches and nodes as compared to plants grown using PANST2. However, no significant difference (p>0.05) was found between their root lengths.

Table 40: Number of branches, nodes, and root length for PANST1 and PANST2

Characteristic	PANST1	PANST2
Number of branches	9.1 ± 0.9^a	8.5 ± 0.3^b
Number of nodes	3.5 ± 0.1^a	3.2 ± 0.2^b
Root length (cm)	13.5 ± 0.7^a	13.9 ± 0.8^a

(n = 42, data indicating different superscript letters across the columns in the same row
are significantly different, one-way ANOVA, p<0.05)

The difference in the basil plants for number of branches and nodes could be due to
higher EC of PANST1 after day 8 than PANST2. The higher EC correlates to more concentration
nutrients especially total nitrogen and nitrates. More nitrates present in PANST1 could have led
to more plant growth. However, previous research on basil morphology and nitrogen dose was
not able to relate the relationship between the two. All the research focused on a fixed amount
of total nitrogen in the soil at the start of the basil growth period. Thus, as the plants grew, total
nitrogen decreased. Walters and Currey (2018) used NFT hydroponic technique to see the effect
of increasing EC (0.5 - 4 mS/cm) on different basil cultivars. Even with use of hydroponic system,
the effect of increasing EC on basil plant morphology was not significant. Use of NFT method to
grow basil led to decease in the EC after 3 weeks growing period. The decrease in the EC
correlated to less available nutrients especially nitrogen as the days passed unlike in PANST1
and PANST2. Another difference between Walter and Currey's experimental set up and our
research was the increase in the EC was achieved by increasing total nutrient content. In this
research, the increase in the EC of PANST1 and PANST2 was due to increase in total nitrogen.

3.3.3.3 Leaf index

From the Table 41, it can be seen that width and leaf index of basil plants grown using
PANST2 was significantly more (p<0.05) than PANST1. There was no significant difference
(p>0.05) between the length of basil leaves grown using the two treatments.

Table 41: Length, width, and LI of basil leaves grown using PANST1 and PANST2

Characteristic	PANST1	PANST2
L (cm)	10.0 ± 0.7 [a]	9.95 ± 0.71 [a]
W (cm)	7.5 ± 0.8 [a]	8.32 ± 0.5 [b]
LI (cm^2) = 0.8 LW	60.7 ± 8.9 [a]	66.52 ± 8.34 [b]

(n = 30, data indicating different superscript letters across the columns in the same row are significantly different, one-way ANOVA, p<0.05)

Nitrates are the signaling molecules involved in leaf morphogenesis. The more available nitrates could also lead to increased uptake of Mg causing more photosynthesis. But in this research the significant increase in leaf index of PANST2 treated basil leaves was not due to presence of more nitrates as PANST1 had more nitrates throughout the harvest period of 21 days. The reason for increased leaf index could be use of fresh PANST2 for week 2 and week 3. The fresh PANST2 had optimum EC especially total nitrogen. The optimum level of nutrients at the start of week 2 and week 3 could have led to their better absorption by the basil plant roots resulting in significant increase in the leaf index. However, no such increase was observed in the PANST2 plant height, number of branches, and nodes as seen previously and in fresh and dry weight of the plants explained in section 3.3.8. One possible explanation would be to compensate for the less available total nitrogen than PANST1, PANST2 plants produced less number of leaves as seen by Sifola et al. (2006). Thus, even though the leaf index for PANST2 plants was significantly higher, number of leaves were less than PANST1. This explanation can be

supported by the fact that fresh and dry weight of PANST1 basil plants was significantly more than PANST2 (section 3.3.8).

3.3.4 Comparison of basil leaf color
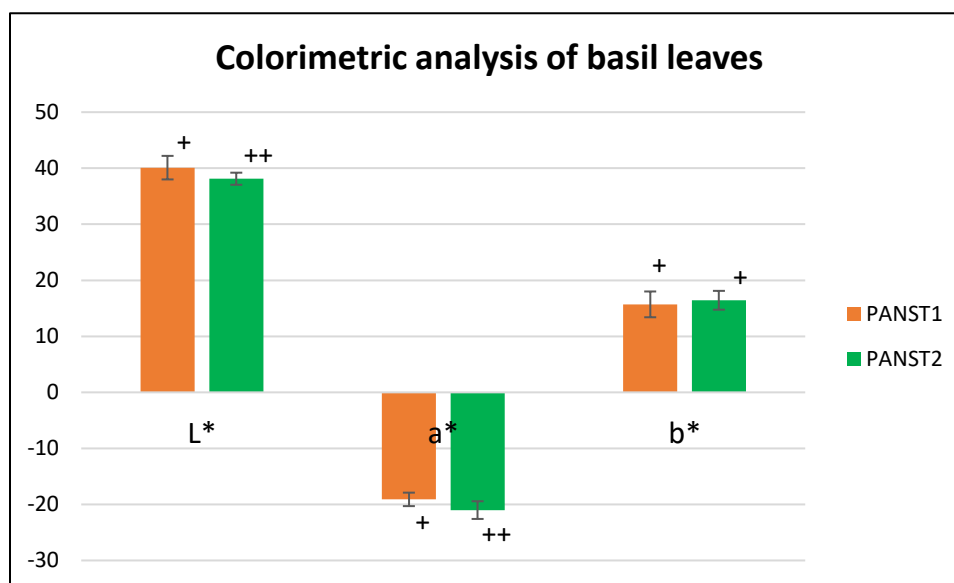
Figure 83: Basil leaf color for plants grown using PANST1 and PANST2 (error bars indicate standard deviation, n = 30, data not sharing the same number of symbols (+) are significantly different from each other (one-way ANOVA, $p < 0.05$).

L* and a* color values were significantly different for basil leaves grown using PANST1 and PANST2. No significant difference was found between their b* color value (Fig. 83). Basil leaves grown using PANST2 were darker in color (significantly lower L* value) and were greener (significantly higher a* value) than PANST1 leaves. This could be due to better utilization of nitrates in PANST2 due to use of fresh PANST2 to water the plants for week 2 and week 3 which could have led to more synthesis of chlorophyll resulting in greener leaves. Also, as discussed in

section 3.3.3.3, the total number of leaves per plant for PANST2 might be less than PANST1 which could have affected the nitrate utilization for chlorophyll synthesis.

3.3.5 Comparison of basil leaf texture

As seen in Fig. 84 the peak rupture force required to puncture the basil leaves was not significantly different for basil leaves grown using PANST1 and PANST2. No significant difference was observed in the Young's modulus (Fig. 85) which is a measure of leaf elasticity. Toughness of the basil leaves too was not significantly different (Fig. 86). Thus, there was no significant difference between the texture of basil leaves grown using PANST1 and PANST2. Hence, use of fresh PANST2 for week 2 and week 3 and increasing EC of PANST1 throughout the harvest period did not cause any difference in the basil leaf texture between them.

Peak rupture force of basil leaves

PANST1: a, 0.8 ± 0.1
PANST2: a, 0.76 ± 0.1

Force (N), y-axis from 0 to 1.2
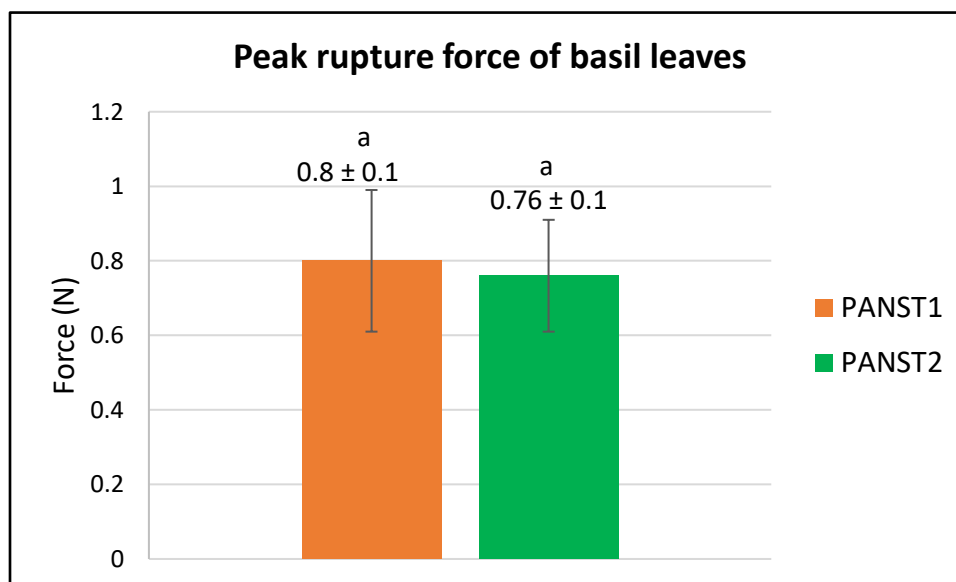
Figure 84: Peak rupture force of basil leaves grown using PANST1 and PANST2 (error bars indicate standard deviation, n = 30, data not sharing the same letters are significantly different from each other, one-way ANOVA, p<0.05).
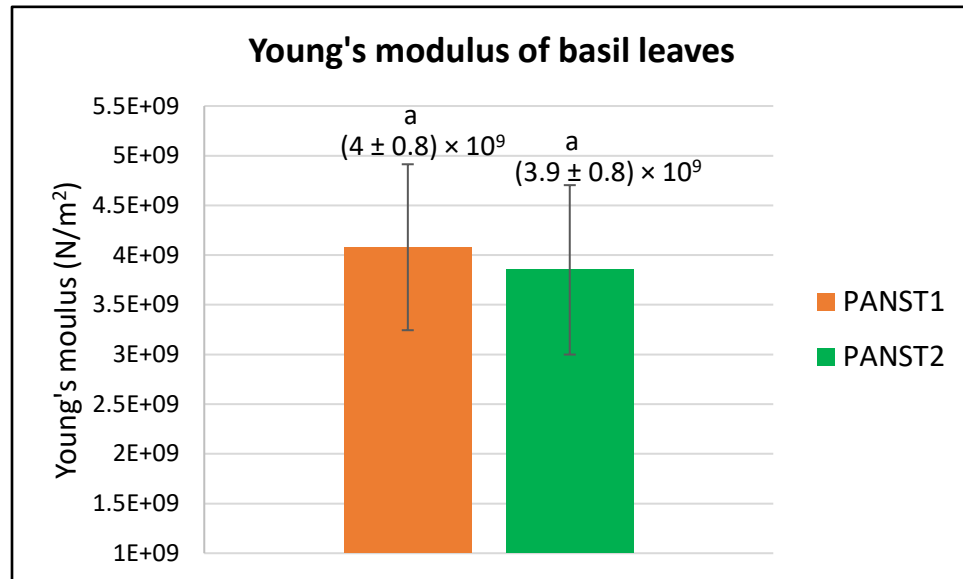
Figure 85: Young's modulus of basil leaves grown using PANST1 and PANST2 (error bars indicate standard deviation, n = 30, data not sharing the same letters are significantly different from each other, one-way ANOVA, p<0.05).
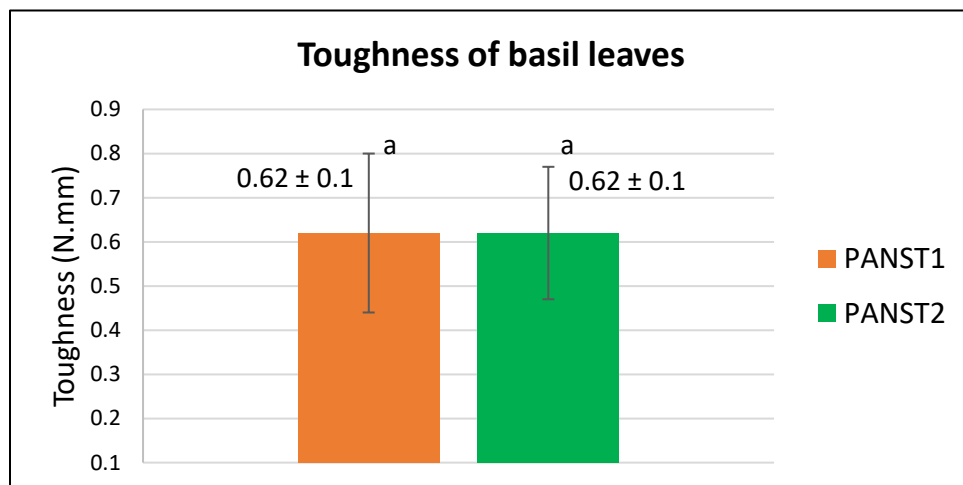
Figure 86: Toughness of basil leaves grown using PANST1 and PANST2 (error bars indicate standard deviation, n = 30, data not sharing the same letters are significantly different from each other (one-way ANOVA, p<0.05).

3.3.6 Comparison of algae content
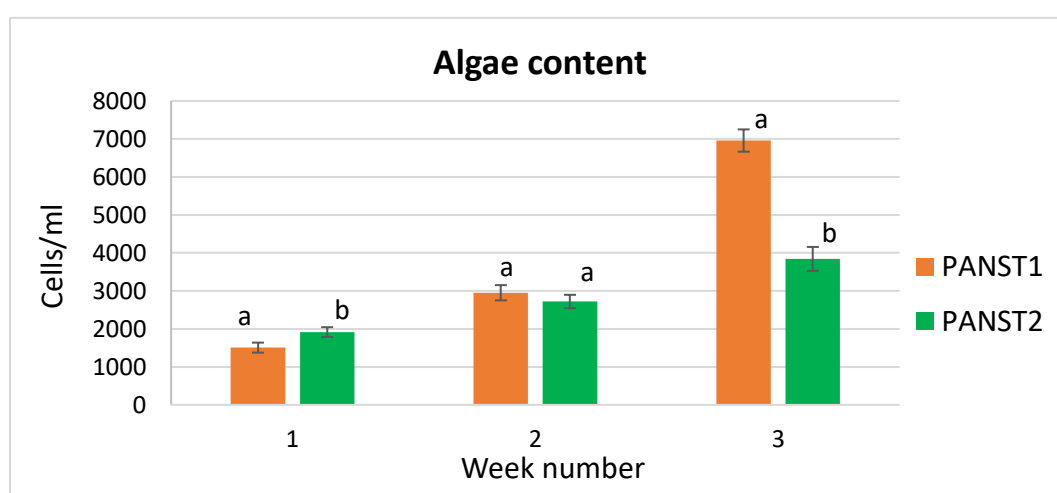
Figure 87: Algae concentration in PANST1 and PANST2 in weeks 1, 2, and 3 (error bars indicate standard deviation, n = 9, data not sharing the same letters are significantly different from each other, one-way ANOVA, p<0.05).

The algae concentration was measured at the end of every week. As seen in the Fig. 87 PANST2 had significantly more algae than PANST1 at the end of week 1. The nitrate and nitrite concentrations on day 1 for PANST1 and PANST2 were (191.9 ± 3.1) ppm, (18.8 ± 1.9) ppm and (189.8 ±1.7) ppm, (18.5 ± 0.9) ppm, respectively. Thus, the amount RNS cannot be the reason for significantly less algae in PANST1. PANST1 treatment was done before PANST2, and the flood tables, tanks, and pumps did not have any algae beforehand. Only possible reason for more

algae in PANST2 for week 1 could be improper cleaning of flood tables, tanks, and/or water pumps resulting in leftover algae cells. For end of the week 2, there was no significant difference between the algae content of PANST1 and PANST2. Ideally, the algae content of PANST2 at the end of week 2 should be comparable to algae content of PANST2 at the end of week 1, because for week 2 fresh PANST2 was used. But the increased algae growth was due to transfer of algae cells from the flood table and water pump to fresh PANST2. This transfer was possible as only the tank was cleaned before adding fresh PANST2 in it. The flood table, water pump, and pipes were not cleaned and had algae growth on them. For end of week 3, the algae content of PANST1 was significantly more than PANST2. This was because of use of same PANST1 for 21 days and use of fresh PANST2 for week 3. The algae continued to grow in PANST1 over 21 days. The effects of RNS decreased with time leading to less algae removal. The fresh PANST2 did not have any algae at the start of week 3. Same as that for week 2, ideally the algae concentration of PANST2 at the end of week 3 should be comparable to PANST2 algae concentration at the end of week 1 as fresh PANST2 was used to water the plants for week 3. But the increased concentration for week 3 as compared to week 1 and week 2 was due to continual algae growth in the flood table, pipes and pumps. However, it can be noticed from Fig. 87 that the algae concentration of PANST2 was close to half of that of PANST1 at the end of week 3.

3.3.7 Comparison of microbial quality

Total plate count (TPC) of basil leaves grown on PANST1 was 3.5 ± 0.07 CFU/g and for PANST2 for was 3.4 ± 0.1 CFU/g with a detection limit of 3.1 log CFU/g and no significant difference was observed between the plate counts of PANST1 and PANST2 basil leaves.

3.3.8 Comparison of basil yield

From Table 42 it can be seen that the fresh and dry weight of basil plants grown using PANST1 were significantly more than PANST2. No significant difference was found in the moisture content between the two treatments.

Table 42: Fresh, dry weight, and moisture content (w.b.) of basil plant grown using PANST1 and PANST2

Characteristic	PANST1	PANST2
Fresh weight (g)	40.3 ± 1.4 [a]	37.6 ± 2.4 [b]
Dry weight (g)	3.8 ± 0.2 [a]	3.4 ± 0.3 [b]
Moisture (%)	90.5 ± 0.8 [a]	90.7 ± 0.8 [a]

(n = 9, data indicating different superscript letters across the columns in the same row are

significantly different, one-way ANOVA, $p<0.05$)

The gradual increase in the EC of PANST1 over the harvest period of 21 days was more than PANST2 after day 8. This was due to use of fresh PANST2 for week 2 and week 3 which resulted in comparatively lower EC than PANST1. The significant difference between fresh and dry weight of basil plants could be because of more available nutrients, especially total nitrogen and nitrates to basil plants grown using PANST1.

3.3.9 Aroma analysis

From the aroma profile (Table 43) it can be seen that PANST2 had significantly more linalool % than PANST1, and significantly less eugenol and methyl eugenol.

Table 43: Composition of basil leaves essential oil for Treatment 2

Aroma compound	PANST1	PANST2
Linalool (%)	69.5 ± 5 [a]	80 ± 2.3 [b]
Eucalyptol (%)	9.7 ± 2.8 [a]	8.7 ± 0.8 [a]
Eugenol (%)	0.5 ± 0.3 [a]	0.07 ± 0.02 [b]
Methyl eugenol (%)	1.5 ± 0.5[a]	0.8 ± 0.2 [b]

(n = 9, data indicating different superscript letters across the columns in the same row are significantly different, one-way ANOVA, $p < 0.05$)

The significantly less eugenol and methyl eugenol in PANST2 could be due less available total nitrogen as compared to PANST1 plants. The increase in linalool content of PANST2 cannot be explained based on availability of total nitrogen, as PANST1 basil plants had more available total nitrogen throughout 21 days. However, this discrepancy could be due to the time taken between the drying of basil samples and aroma analysis by GCMS. For PANST1, the time between drying of the samples and actual analysis was a month more than PANST2 samples.

3.3.10 Comparison of tissue nutrient content of basil leaves

From Table 44, it can be seen that nutrients N, P, K, Cu, and B were higher in basil leaf tissue grown using PANST1 than PANST2. Ca, Mg, S, and Mn tissue nutrient levels were comparable between the two treatments. And Fe level in basil leaves grown using PANST1 was less than PANST2.

Table 44: Tissue analysis data for basil leaves grown using PANST1 and PANST2 along with sufficiency range of nutrients for basil

Nutrients	PANST1	Sufficiency range	PANST2
N%	6.4 ± 0.1	4 – 6	6 ± 0.2
P%	0.82	0.62 - 1	0.6 ± 0.02
K%	6.6 ± 0.2	1.55 - 2.05	5.7 ± 0.3
Ca%	2.5 ± 0.1	1.25 - 2	2.4 ± 0.1
Mg%	0.5 ± 0.02	0.6 – 1	0.5 ± 0.03
S%	0.45 ± 0.02	0.2 – 0.6	0.4 ± 0.04
Mn (ppm)	36.9 ± 5.8	30 – 150	39.4 ± 3.1
Zn (ppm)	38.8 ± 0.3	30 – 70	36.3 ± 14.2

Cu (ppm)	12.8 ± 0.8	5 - 10	11.6 ± 3.2
B (ppm)	54.3 ± 5.5	25 - 60	41.5 ± 3.2
Fe (ppm)	57.7 ± 56.2	75 – 200	95.4 ± 6.7

EC of PANST1 gradually increased with time and was higher than PANST2 after day 8. This implies more availability of nutrients to basil plants grown using PANST1. This could be the reason for increased level of nutrients N, P, K, Cu, and B in basil leaves grown using PANST1 as compared to PANST2.

3.4 Comparison of basil plants grown using NST (6 batches), PANST1 (3 batches) and PANST2 (3 batches)

In this section, to get an overall picture of how the two plasma treatments affected the main quality and growth parameters of basil plants, the combined data from the control (NST1 and NST2) will be compared with the plasma (PANST1 and PANST2) treatments.

3.4.1 Plant height analysis

As seen in Fig. 88, the two plasma treatments resulted in taller basil plants at the end of the 21 days. However, as seen in section 3.3.3.1, average basil plant height after PANST1 was higher than control (NST) and PANST2 by 11 % and 5 %, respectively.
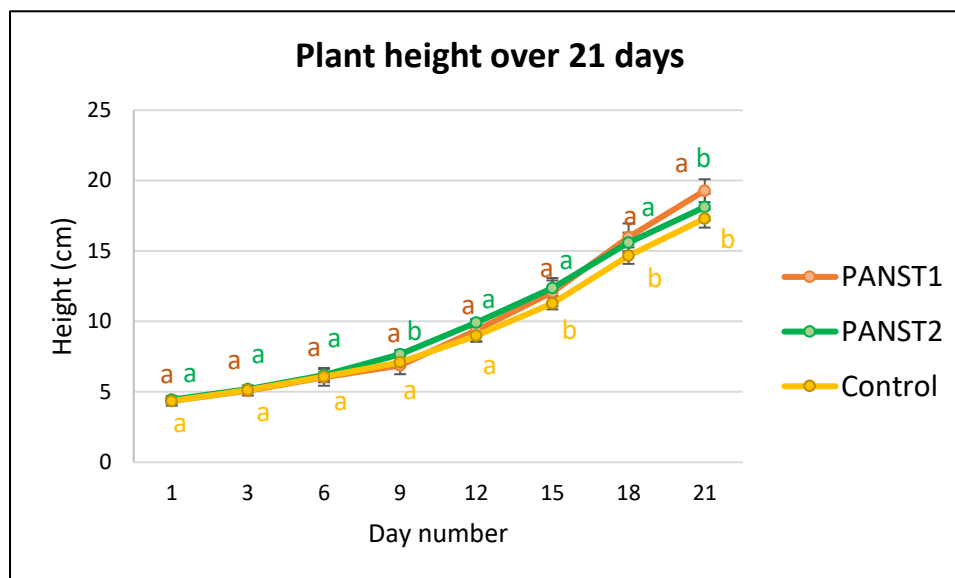
Figure 88: Plant height analysis of two plasma treatments and control over 21 days (error bars indicate standard deviation, n = 42 * 2 for control, n = 42 for PANST1, n = 42 for PANST2, data not sharing the same letters on the given day are significantly different from each other, one-way ANOVA, $p < 0.05$).

3.4.2 Number of branches, nodes, and root length

From Table 45, it can be seen that the number of branches and nodes were significantly higher for PANST1 plants compared to control and PANST2. The root length of both the plasma treatments was significantly less than the control basil plants.

Table 45: Number of branches, nodes, and root length for control and plasma treatments

Characteristic	Control	PANST1	PANST2
Number of branches	8.6 ± 0.6^a	9.2 ± 0.9^b	8.5 ± 0.3^a
Number of nodes	3.3 ± 0.1^a	3.5 ± 0.3^b	3.2 ± 0.2^a
Root length (cm)	15.3 ± 0.5^a	13.5 ± 0.7^b	13.9 ± 0.8^b

(n = 42 * 2 for control, n = 42 for PANST1, n = 42 for PANST2, data indicating different superscript letters across the columns in the same row are significantly different, one-way ANOVA, $p<0.05$)

3.4.3 Leaf index

The leaf index of control and PANST1 was not significantly different from each other. But the leaf index of PANST2 was significantly more than control and PANST1.

Table 46: Length, width, and leaf index for control, PANST1, and PANST2

Characteristic	Control	PANST1	PANST2
L (cm)	9.3 ± 0.8^a	9.9 ± 0.7^a	9.9 ± 0.7^a
W (cm)	7.6 ± 0.8^a	7.6 ± 0.9^a	8.3 ± 0.5^b
LI (cm^2) = 0.8 LW	57.7 ± 9.6^a	60.5 ± 9.5^a	66.5 ± 8.3^b

(n = 30 * 2 for control, n = 30 for PANST1, n = 30 for PANST2, data indicating different

superscript letters across the columns in the same row are significantly different, one-way

ANOVA, $p < 0.05$).

3.4.4 Leaf Color

As seen in Fig. 89, L* value was not significantly different between control and PANST2

treatments and for PANST1 treatment the L* value was significantly more. The a* value for both

the plasma treatments was significantly lower than the control implying that plasma treatment

led to more greener leaves. The b* value of both the plasma treatments was significantly less

than the control implying that the control leaves were more yellow. The use of PANST2 led to

more green and less yellow leaves when compared to leaves grown using PANST1.
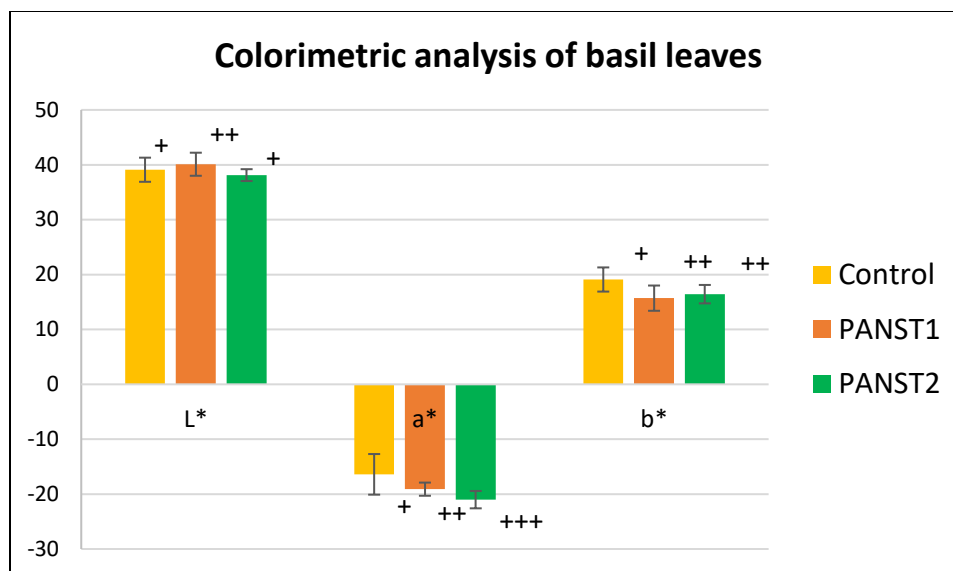
Figure 89: Colorimetric analysis of two plasma treatments and control (error bars indicate standard deviation, n = 30 * 2 for control, n = 30 for PANST1, n = 30 for PANST2, data not sharing the same number symbols (+) are significantly different from each other, one-way ANOVA, p<0.05).

3.4.5 Texture

On comparison of peak rupture force, leaf toughness, and Young's modulus of the basil leaves, there was no significant difference between control, and two plasma treatments (Table 47). Hence both the use of plasma treatments did not affect the basil leaf texture.

Table 47: Peak rupture force, leaf toughness, and Young's modulus of control and plasma treatments

Characteristic	Control	PANST1	PANST2

Peak rupture force (N)	0.7 ± 0.2^a	0.8 ± 0.2^a	0.8 ± 0.2^a
Toughness (N.mm)	0.6 ± 0.2^a	0.6 ± 0.3^a	0.6 ± 0.2^a
Young's modulus (N/m^2)	$(3.9 \pm 0.8) \times 10^{9\,a}$	$(4 \pm 0.8) \times 10^{9\,a}$	$(3.9 \pm 0.8) \times 10^{9\,a}$

(n = 30 * 2 for control, n = 30 for PANST1, n = 30 for PANST2, data indicating different superscript letters across the columns in the same row are significantly different, one-way ANOVA, p<0.05).

3.4.6 Algae concentration

As seen in Fig. 90, at the end of week 1 and week 2, the algae content of control was significantly higher than both the plasma treatments. However, by the end of week 3, there was no difference in the algae content of control and PANST1 but the PANST2 had significantly less algae than control and PANST1. This was due to use of same PANST1 to water the plants for 3 weeks and use of fresh PANST2 for week 3.
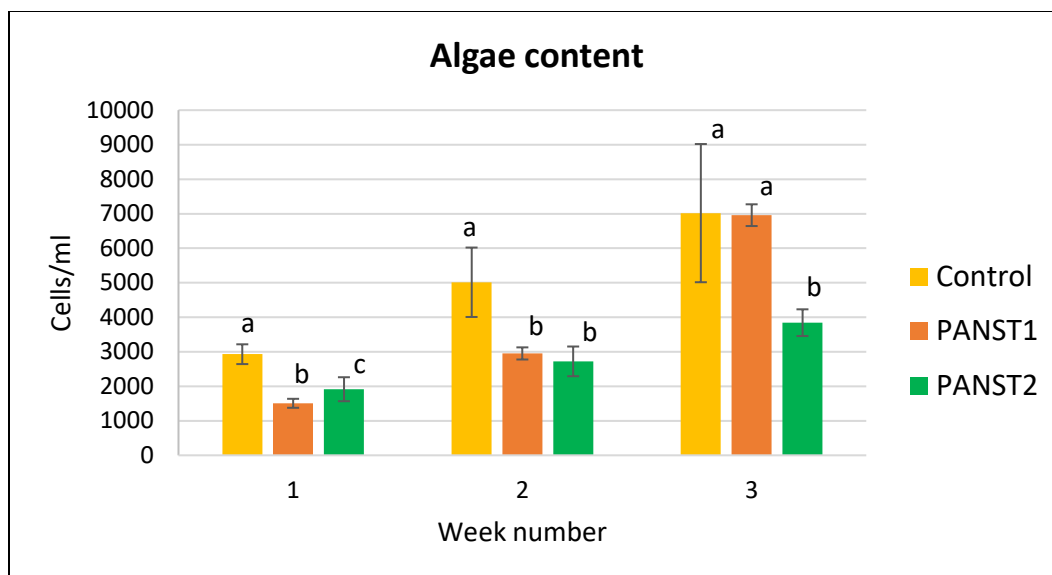
Figure 90: Algae analysis of two plasma treatments and control at the end of every week (error bars indicate standard deviation, n = 9 * 2 for control, n = 9 for PANST1, n = 9 for PANST2, data not sharing the same letters are significantly different from each other, one-way ANOVA, p<0.05).

3.4.7 Fresh and dry weight analysis

For fresh and dry weight of the basil plants (Table 48), the control had significantly less fresh and dry weight than both the plasma treatments and there was no significant difference between their moisture content.

Table 48: Average fresh weight, dry weight, and moisture content (w.b.) of control and plasma

treatment basil plants

Characteristic	Control	PANST1	PANST2
Fresh weight (g)	31.1 ± 3.2 [a]	40.18 ± 1.6 [b]	37.6 ± 2.4 [c]
Dry weight (g)	2.8 ± 0.4 [a]	3.8 ± 0.2 [b]	3.4 ± 0.3 [c]
Moisture (%)	91.4 ± 0.7 [a]	90.4 ± 0.9 [a]	90.7 ± 0.8 [a]

(n = 9 * 2 for control, n = 9 for PANST1, n = 9 for PANST2, data indicating different superscript

letters across the columns in the same row are significantly different, one-way ANOVA,

$p<0.05$).

3.4.8 Aroma analysis

The aroma analysis was performed using a GC to evaluate the relative abundance of

basil leaf aroma compounds (Fig. 91, Fig. 92, and Fig. 93). From the essential oil profile (Table

49), it can be seen that basil leaves grown using PANST2 had significantly more linalool than

leaves grown using PANST1 and control. The basil leaves grown using PANST1 had significantly

more methyl eugenol than leaves grown using control and PANST2, and there was no significant

difference between the profile of eucalyptol and eugenol.
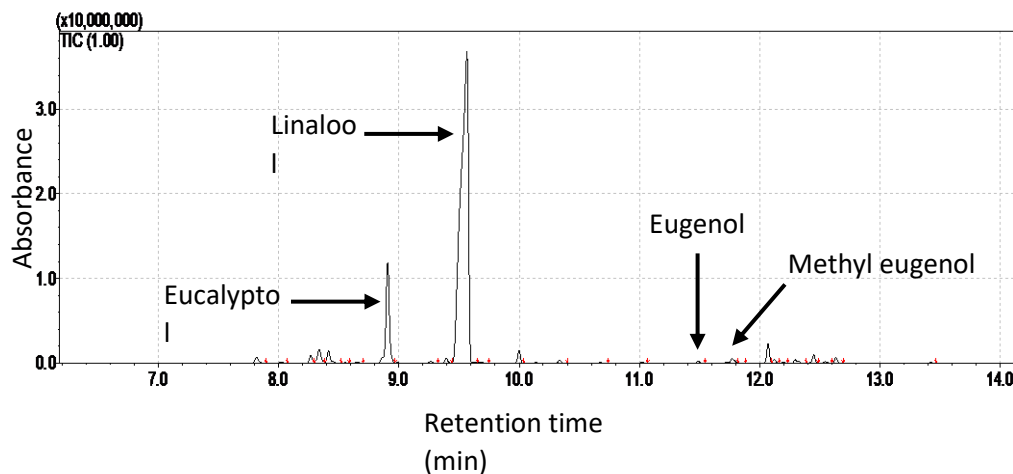
Figure 91: Aroma analysis chromatogram showing relative abundance of aroma compounds for control basil leaves analyzed on Shimadzu 2010 Plus gas chromatograph equipped with an AOC-6000 auto-sampler using a H-Rxi-5Sil MS column held at 35 °C for 4 min.
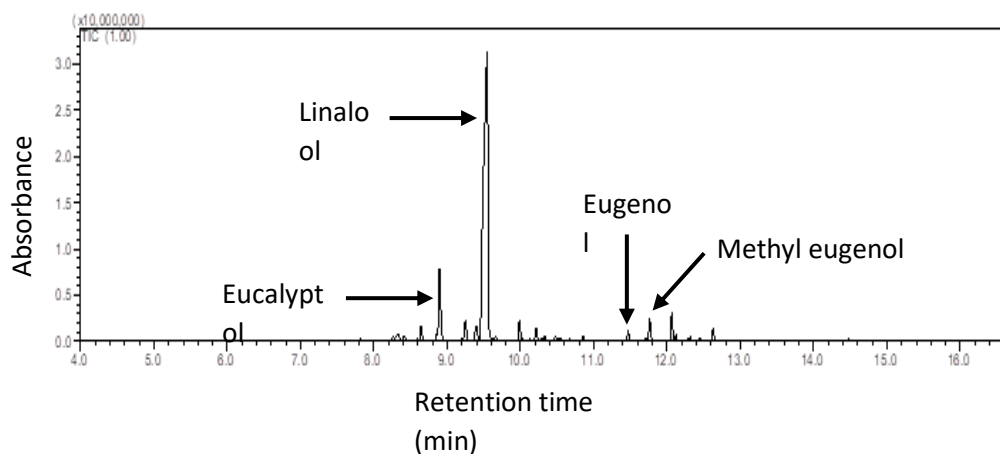
Figure 92: Aroma analysis chromatogram showing relative abundance of aroma compounds for basil leaves grown using PANST1 analyzed on Shimadzu 2010 Plus gas chromatograph equipped with an AOC-6000 auto-sampler using a H-Rxi-5Sil MS column held at 35 °C for 4 min.
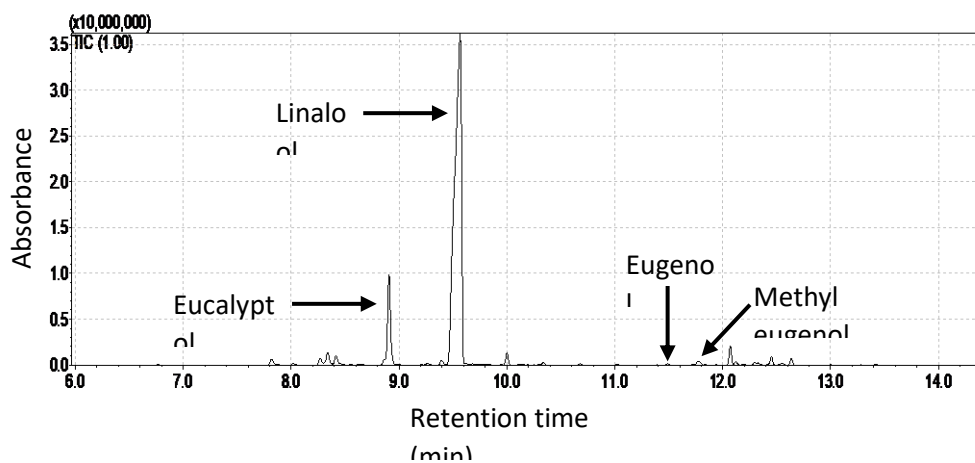
Figure 93: Aroma analysis chromatogram showing relative abundance of aroma compounds for basil leaves grown using PANST2 analyzed on Shimadzu 2010 Plus gas chromatograph equipped with an AOC-6000 auto-sampler using a H-Rxi-5Sil MS column held at 35 °C for 4 min.

Table 49: Composition of basil leaves essential oil for control and two plasma treatments

Aroma compound	Control	PANST1	PANST2
Linalool (%)	68.8 ± 11.6 [a]	69.5 ± 5 [a]	80 ± 2.3 [b]
Eucalyptol (%)	10.6 ± 3.8 [a]	9.7 ± 2.8 [a]	8.7 ± 0.8 [a]
Eugenol (%)	0.2 ± 0.2 [a]	0.5 ± 0.3 [a]	0.07 ± 0.02 [a]
Methyl eugenol (%)	0.5 ± 0.1 [a]	1.5 ± 0.5 [b]	0.8 ± 0.2 [c]

(n = 6 + 9 for control, n = 6 for PANST1, n = 9 for PANST2, data indicating different superscript letters across the columns in the same row are significantly different, one-way ANOVA, $p < 0.05$).

As explained in section 3.3.9, some of the control samples and PANST1 samples were analyzed a month later than PANST2 samples. This could have led to some changes in the essential oil profile of dried basil leaves.

It can be seen from section 3.1 (Treatment 1) and section 3.2 (Treatment 2) that both the treatments enhanced growth parameters such as plant height and leaf index and quality parameters such as color of the leaves and basil yield while not affecting its microbial quality and leaf texture. As seen from section 3.3 and section 3.4, use of PANST1 was better in terms of enhancing basil plant height, yield and aroma profile and use of PANST2 was better in terms of enhancing leaf color, improving leaf index, and causing significant algae reduction throughout the harvest period.

4. CONCLUSIONS

A laboratory scale hydroponic system for growing sweet basil using plasma activated nutrient solution (PANS) was designed and successfully developed. It was found that growing basil plants from Rutgers DMR seeds using PANS showed enhanced growth and improvements in some quality parameters of basil leaves, on day 21, which was the harvest day. Two different plasma treatments were considered. Basil grown using standard nutrient solution (NS) was used as control.

For Treatment 1 (PANST1), the PANS and NS (NST1) were generated on day 1 of the growth period and was reused for the rest of the growth period. For Treatment 2 (PANST2), the PANS and NS (NST2) were generated on day 1 of the growth period and were used only in week 1. Fresh PANS and NS were used in week 2 and again in week 3. Basil growth using PANST1 showed significantly more plant height (8 %) and leaf index (6 %) when compared to basil plants grown using NST1. There was no significant difference in the number of branches and nodes of the basil plants between the PANST1 and NST1. However, the root length of basil plants grown using PANST1 was significantly less than plants grown using NST1. The leaves of basil plants grown using PANST1 were significantly greener (higher a* value) and less yellow (lower b* value) in color than the leaves grown using NST1. There was a significant visual color difference between the leaves indicated by their ΔE^*_{ab} value. The basil leaf texture in terms of its peak rupture force, leaf toughness, and Young's modulus was not significantly different between PANST1 and NST1. For the microbial quality of the basil leaves, no significant difference was found between leaves grown using NST1 and PANST1 indicated by their TPC of 3.5 ± 0.06 log CFU/ml and 3.5 ± 0.07 log CFU/ml, respectively. The aroma profile of the basil showed that PANST1 resulted in significantly more linalool (19 %) compared to NST1. Also, presence of

methyl eugenol was detected in basil grown using PANST1 which might indicate spicier taste and aroma of the product. The nutrient analysis of basil leaves tissue showed no significant difference between NST1 and PANST1. PANST1 solution had 47 %, 50 %, and 22 % less algae at the end of weeks 1, 2, and 3, respectively, compared to NST1 solution.

The effect of PANST2 on basil plant growth and quality parameters was similar to that of PANST1. Basil plants grown using PANST2 were significantly taller (7 %) with higher leaf index (12 %) as compared to basil plants grown using NST2. There was no difference in the number of branches and nodes of the basil plants between PANST2 and NST2. However, the root length of basil plants grown using PANST2 was significantly less than the roots of basil plants grown using NST2. The leaves of basil plants grown using PANST2 were significantly greener (higher a* value) and less yellow in color (lower b* value) than the leaves of basil plants grown using NST2. There was visual total color difference between the leaves indicated by their ΔE^*_{ab} value. The basil leaf texture was not significantly different between leaves grown using PANST2 and NST2. For the microbial quality of the basil leaves, no significant difference was found between leaves grown using NST2 and PANST2 indicated by their TPC of 3.3 ± 0.1 log CFU/ml and 3.4 ± 0.1 log CFU/ml, respectively. The aroma profile of the basil too was not significantly different between leaves grown using PANST2 and NST2. The nutrient analysis of leaf tissue of basil plants grown using NST2 and PANST2 showed comparable micro and macronutrients. PANST2 resulted in 35 %, 33 %, and 24 % less algae at the end of weeks 1, 2, and 3, respectively compared to NST2.

For both PANST1 and PANST2 treatments, enhanced growth and improved quality parameters obtained for basil was due to the presence of more nitrates in the solution as compared to NST1 and NST2, respectively. This was confirmed using assays and test strips. Also,

nutrient analysis data of the solutions indicated presence of more nitrates in plasma treated

solutions at the start and at the end of the growth period.

To understand the effect of two different treatments (PANST1 and PANST2), their

results were compared with control (NST1 + NST2) for basil plant growth and quality

parameters, measured on day 21. Basil plants grown using PANST1 were significantly taller (11

%) compared to control and also compared to PANST2 (5 %). The number of branches and nodes

for basil plants grown using PANST1 were significantly more than control and PANST2 plants.

The fresh and dry weight of the basil plants grown using PANST1 were significantly higher than

plants grown using control (28 % and 35 %, respectively) and PANST2 (20 % and 21 %,

respectively). The leaf index of basil leaves grown on PANST2 was significantly more than control

(15 %) and PANST1 (4 %). The leaf color too was greener for leaves grown using PANST2 as

compared to control and PANST1. The texture of the leaves was not affected by the use of

PANST1 and PANST2. There was no significant difference between the microbial quality of the

basil leaves grown using PANST1, PANST2, and control. The aroma profile of basil leaves grown

using PANST2 and control was similar to each other except for the significantly more linalool

present in leaves grown using PANST2. However, the basil leaves grown using PANST1 had

significantly more methyl eugenol and more eugenol indicating spicier taste. For algae, use of

PANST2 caused significant reduction at the end of every week. However, use of PANST1 resulted

in significant reduction at the end of first two weeks only.

Thus, it can be seen that use of PANST1 resulted in improved plant height, number of

branches, nodes, aroma profile, and yield of the basil. The use of PANST2 resulted in improved

leaf index, leaf color, and algae reduction from the system. Hence, use of plasma treatment of

NS can be used in hydroponic plant growth of basil and can prove beneficial for other

hydroponic produce such as lettuce and tomato.

5. FUTURE WORK

In this study, for Treatment 2, fresh PANS was used at the start of weeks 2 and 3 resulting in more use of water. In future, the same PANS used for week 1 could be treated using gliding arc plasma at the start of week 2 and week 3 to check its efficacy.

For Treatment 1 and Treatment 2, the actual % water used by plants to grow was close to 2 %, implying that there was lot of water evaporation from the flood tables. This evaporation could be controlled by using Styrofoam (grodan blocks with basil plants can be placed in the holes made in Styrofoam sheet) which could limit the exposure of water in the flood table to light and could drastically lower the water used by our hydroponic set up.

In future, sensory evaluation (aroma, color, maybe even taste) of the basil leaves grown using NS and PANS can be done to understand differences, if any, in their sensory profiles.

Also, using cold atmospheric plasma to treat basil seeds before germination should be evaluated. There is a lot of promising research on seeds, their plasma treatment, and how it affects their germination and growth. Instead of using PANS to water the plants, basil seeds can be plasma treated and then can be grown by feeding NS to evaluate seedling growth, plant growth, and quality.

Since this research has shown some promise, it can be scaled up to a small greenhouse level for hydroponic production of basil.

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