EFFECT OF NITROGEN AND PLANT DENSITY ON YIELD AND POST-HARVEST QUALITY OF BASIL AND ROCKET

By

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DECLARATION

I, Rebecca Irene Sindisiwe Mahlangu, declare that the research reported in this thesis, for the degree of Ph.D. (Agriculture) at UNISA is my own work, except where duly acknowledged. There has been no submitting or publishing of this thesis for any degree or examination at any other university.

Signed ……………………… Date ………………………..

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ABSTRACT

Basil *(Ocimum basilicum* L.) and rocket (*Diplotaxis tenuifolia* (L.) DC.) are an important part of the human diet and consumed as herbs and vegetables. The cosmetic and pharmaceutical industries also use these herbs, and may help to prevent various forms of human diseases such as pancreas and colon cancer. In agricultural practice, the quality and yield of many herbs or vegetables depend largely on fertiliser application and plant density. Nitrogen (N) application is essential for growing healthy leafy vegetables, as it influences the green photosynthetic pigment, chlorophyll content, and marketable yield of herbs. Nutrient elements such as N, which is the basic component of chlorophyll, affect photosynthesis capacity. Poor management in plant spacing and N fertiliser application on high-value herbs, such as basil and rocket, can reduce the plant growth, yield, quality, and phytochemicals content. Basil is a popular culinary herbal crop grown for its fresh or dry leaf. It contains phytochemicals properties such as flavonoids and volatile terpenes, such as camphor and linalool. Known as a salad crop and for its healing properties, rocket contains phytochemicals properties, vitamin C and carotenoids. The N exerts its influence on vegetative aerial parts, and primary and secondary plant metabolites accumulation. There are few reports on the effect of N fertiliser application and plant density on yield, phytochemical content and antioxidant properties of basil and rocket during post-harvest storage, which are important quality aspects of herbs for human health.

This study investigates the effects of N application (60, 90, 120, 150, and 180 $kg{\cdot}ha^{-1}$), plant density (40,000; 62,500 and 93,750 $plants{\cdot}ha^{-1}$ for basil; 40,000; 80,000 and 133,333 $plants{\cdot}ha^{-1}$ for rocket), and post-harvest storage duration (0, 5, 10, or 15 days) on yield, phytochemical and antioxidant properties of basil and rocket. The arrangements of treatments were 5 × 3 × 4 factorial combinations in a randomised complete block design replicated three times. Parameters measured were fresh leaf mass, dry leaf mass, leaf area, fresh shoot mass, dry shoot mass and leaf chlorophyll content. The post-harvest quality, such as parameters, total phenolic content (TPC), flavonoids content (FC), free radical scavenging activity, and antioxidant activity were analysed. Leaf chlorophyll content of basil and rocket improved with the application of N at 120 kg·ha^{-1}. Fresh leaf mass and area increased with increased plant density for both crops. Plant density at 40,000 plants·ha^{-1} for rocket, produced high K leaf content at 120 kg·ha^{-1} of N. Leaf N, calcium (Ca) and Magnesium (Mg) contents for basil improved at 62,500 or 93,750 plants·ha^{-1}. N application at 60–180 kg·ha^{-1} did not affect leaf yield of basil and rocket. Application of 60 kg·ha^{-1} N at 93,750 plants·ha^{-1} for basil, and 60 kg·ha^{-1} of N at 133,333 plants·ha^{-1} for rocket, improved leaf yield. Nitrogen application of 120 kg·ha^{-1} at day 0 of storage caused the highest accumulation of TPC, TFC, strong-free radical scavenging activity and antioxidant activity with limited effect by plant density on basil. In rocket, the application of 60 to 120 kg·ha^{-1} N at day 0 (freshly harvested) of storage had high TPC, while TFC was high at 90 to 180 kg·ha^{-1} N and 10 days of storage. Rocket had strong scavenging activity at 120 to 180 kg·ha^{-1} N at 15 days of storage, and for 60 and 90 kg·ha^{-1} N at 0 and 10 days of storage. Post-harvest quality of basil was sensitive to storage and high N application. TPC, TFC and free radical scavenging activity sagged continuously with the extension of storage days. The combination of 120 kg·ha^{-1} at day 0 of storage of basil, and for the rocket at 60 kg·ha^{-1} N and 10 days of storage were ideal. Spacing had limited response in all parameters recorded; there was no significant difference of phytochemicals on plant spacing. Increasing plant density and providing a reasonable amount of N could be useful to obtain high marketable leaf yield along with efficient N management.

This study concluded and recommended that the most economic treatment for basil was 60 kg·ha^{-1} N for TPC, FC, FRS, and antioxidant activity, while for rocket, it was 120 kg·ha^{-1} with a longer shelf-life of 10 days of storage with regard to antioxidant activity. Optimising agronomic practices for improved production should consider phytochemical quality assurance to ensure there is no compromise in crop health benefits to consumers. The increased marketable yield, particularly at high plant density, may result in an increased profit margin and income generation for the producer.

LIST OF CONFERENCES

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LIST OF SYMBOLS AND ABBREVIATIONS

DPPH	2,2-diphenyl-1-picrylhydrazyl
ATP	Adenosine 5'-triphosphate
ARC-VIMP	Agricultural Research Council - Vegetable, Industrial and Medicinal Plants
AlCl3	Aluminium chloride
NH_4^+	Ammonium nitrogen
ANOVA	Analysis of variance
&	And
AA	Ascorbic acid
B	Beta
B	Boron
BHA	Butylated hydroxyanisole
BHT	Butylated hydroxytoluene
Ca	Calcium
CO_2	Carbon dioxide
cm	Centimeter
Cl	Chlorine
CRBD	Randomised Complete Block Design
Cu	Copper
^oC	Degree Celsius
DNA	Deoxyribonucleic acid

DAFF	Department of Agriculture, Fisheries and Forestry
DDH_2O	Double distilled water
=	Equal
e.g.,	Example
FAO	Food and Agricultural Organization
G	Gram
>	Greater than
ha	Hectare
Fe	Iron
Kg	Kilogram
LSD	Least Significant Difference
<	Less than
L	Litre
Mg	Magnesium
Mn	Manganese
MAP	Modified atmosphere packaging
Mo	Molybdenum
NO_3^-	Nitrate
NO_2^-	Nitrite
N	Nitrogen
O	Oxygen
DW	Dry weight

FW	Fresh weight
%	Percentage
P	Phosphorus
K	Potassium
Pty Ltd	Proprietary Limited
RNS	Reactive nitrogen species
ROS	Reactive oxygen species
RH	Relative humidity
RNA	Ribonucleic acid
Na	Sodium
SPAD	Soil plant analysis development
S	Sulphur
T	Temperature
TFC	Total flavonoids content
TPC	Total plant phenolic content
UNISA	University of South Africa
USDA	United States Department of Agriculture

CHAPTER 1

1.1 General Introduction

Culinary herbs have been an essential component of human consumption, and the growing demand for a constant supply of fresh herbs is driving the market across the world (Raya et al., 2015). Leafy greens (rocket, baby spinach and lettuce) and herbs (basil, mint, and coriander) are in demand in the South African market. Herbs have the potential to add variation, flavour to foods, and preservation of meat (Cantwell & Reid., 1993). The demand for herbal medicines has been growing over the last few decades, owing to public awareness of ‘back to nature' for a healthier life (Raya et al., 2015). Herbs and spices application, culinary, meat and poultry by-products hold approximately 45.6% of the major share of the herbs and spices market, with culinary application holding about 31.3% and meat and poultry products about 14.3% in 2017 (Smith, 2018).

Production of aromatic plants for profit on a commercial basis involves several factors such as cultivar, fertiliser, quality, and plant population. Nutrient management allows efficient and sustainable improvement of herb yield and quality (Corrado et al., 2020). Yield and quality of herbs and leafy greens in terms of nutritional qualities (phytochemical content, antioxidants and flavonoids) can be subject to several key agronomic practices (plant density, and N fertiliser) and growing conditions (Bjorkman et al., 2011; Bonasia et al., 2017; Miceli et al., 2020). Herbs have global importance, not only as a widely employed food garnish, but also as a raw material for phytochemical preparations with proven health benefits. Macronutrient management, particularly for leafy green vegetables, optimising N application is essential to balance valued increase of plant metabolism with a more limited accumulation of nitrates (NO_3^-) in edible organs. Achieving high-quality produce in vegetable production can be difficult due to challenges with controlling NO3- concentration in plant tissue (Corrado et al., 2020).

Basil *(Ocimum basilicum* L.) belongs to the Lamiaceae family, sub-family *Nepetoideae*, and the genus sensu-lato consisting of 65 species (Paton et al., 1999). Basil leaves contain essential oils of distinct aroma and are utilised as fresh and dried to enhance flavour in various meals. Traditionally, the consumption of basil is as a medicinal herb, as a remedy for headaches, diarrhoea, coughs, warts, constipation and kidney malfunction (Politeo et al., 2007; Klimankova et al., 2008). Basil is a source of numerous phytochemical compounds, such as phenol derivatives, terpenoids, flavonoids and phenylpropanoids, with known biological, pharmaceutical and industrial uses (Corrado et al., 2020). Basil is substantial for pharmaceutical and cosmetic preparations due to the high content of phenolic compounds, widely known as phytochemical molecules, found in all plants (Mastaneh et al., 2014). *Ocimum spp.,* consisting of rosmarinic acids (RA), is one of the plentiful caffeic acid esters existing in numerous important biological properties such as antioxidant, antibacterial, antiviral and anti-inflammatory activities (Pereira, 2005; Alagawany, 2017). However, basil leaves regularly deteriorate in quality throughout transportation, and shelf life depends on the environmental conditions (Buchanan-Wollaston, 1997).

Rocket (*Diplotaxis tenuifolia* (L.) DC.) belongs to the family of Brassicaceae. Originally, it was cultivated as an herbaceous plant, but nowadays it is an important leafy vegetable, with cumulative influence in the national and international vegetable markets (Caruso et al., 2019). The leaves of rocket have a pungent taste and are widely utilised to flavour salads; the contrast in flavour is great, depending on genetic diversity, species and environmental condition. Commonly known as a salad crop, rocket also has medicinal properties, containing phytochemicals properties and carotenoids (Padulosi & Pignone, 1996). Rocket is rich in minerals and vitamin C, which is especially effective in many biological activities in the human body (Conesa et al., 2009). Numerous studies on rocket species identified a larger concentration of polyglycosylated flavonol compounds, which belong to the core aglycones such as isorhamnetin, kaempferol and quercetin that contain numerous health benefits in humans and animals (Bell et al., 2015).

Plant nutrition is one of the important factors that increases crop productivity. Nitrogen (N) is the first limiting macronutrient essential for plant growth; it plays a vital role in the different synthesis of the constituents of different plants through the action of several enzymes (Khalid et al., 2018). Nitrogen affects the primary and secondary metabolic pathways, thus secondary plant metabolites accumulation (Chen et al., 2011). Good management of N fertiliser application is necessary because its excessive use can harm the environment through leaching and reduce the quality of green leafy vegetables (Mola et al., 2019). N deficiency can cause biochemical and physiological disturbances leading to a decrease in cell division rates and perturbation in process of photosynthesis. High accumulation of N in plants may cause toxicity problems for human health, such as methemoglobinemia (Elhanafi et al., 2019). Excessive application of N fertiliser could lead to a significant increase in nitrate (NO_3^-) and N leaching in groundwater (Akbariyeh et al., 2018). In normal cultivated soil, NO_3^- is the main form of N obtainable for plant uptake. The belief is that nitrate is a chemical contaminant of fresh produce. An excess of N application might reduce the quality of the leafy vegetables because of the accumulation of NO_3^- (Boink & Speijers, 2001; Cavarianni et al., 2008). Plants can be attacked by pathogens when, excessive N fertilisation triggers superfluous vegetative growth (Mola et al., 2019). Reportedly, increasing N application can increase or decrease plant resistance to pathogens, which reflects differences in the infection strategies of discrete pathogens (Mur et al., 2017). Growers need to control N supply to leafy crops and ensure the risks of diffuse pollution from overuse of N fertiliser are minimised (Weightman & Hudson, 2013). There are limited studies about N fertiliser application at different plant densities on basil and rocket growth, yield and phytochemical compound. There is also a lack of consistent results concerning storage losses and the chemical composition of herbs after N application.

1.2 Research justification

Plant spacing is a crucial aspect in determining the micro-environmental condition in field-grown and protected growing conditions of leafy vegetables and herbs. The optimisation of plant density can lead to a high plant yield, favourably affecting the absorption of nutrients and exposure of the plant to light (Khorshidi et al., 2009). Optimum plant density allows the plants to grow uniformly and suitably through efficient utilisation of moisture (Firoz et al., 2009). Utilising ideal plant density could produce optimum yield, while too low or too high plant density might result in lower quality and yields. Plant spacing certainly affected fresh leaf mass, leaf area, and leaf dry of Swiss chard grown under hydroponics (Maboko & Du Plooy, 2013). Numerous studies have stated that agronomic aspects (e.g., soil types, fertilisers, pesticides, cultivation methods, and habitat manipulations) and environmental conditions (e.g., temperature, season, light, water, humidity, and CO_2), can affect the phytochemicals content in vegetables and herbs (Choi et al., 2018), and phenolics, flavonoids, and other bioactive compounds in broccoli (Pek et al., 2013). Studies have shown that optimal plant spacing has significant effects on the growth and bioactive compound content of watercress (Kaluzewicz et al., 2017; Machado et al., 2018).

Consumer acceptance of aroma, nutrients, taste and presentation of the product define the quality of food. The rapid growth of the ready-to-eat product category increased the demand for convenient foods (Hofstrand, 2008). There is rising attention to natural antioxidants obtainable in plants because of the global trend concerning the usage of natural additives in diet and cosmetics (Yanishlieva et al., 2006). An antioxidant is one of the phytochemical compounds that at low concentration prevents or delays the oxidation of a substrate. The oxidative stress in biological systems is a complex process categorised by an imbalance between the production of free radicals (FR) and the body's ability to eradicate these reactive species by the use of both endogenous and exogenous antioxidants (Santos-Sanchez et al., 2019). Phenolic acids have attained substantial attention as possible protective aspects against heart diseases and cancer due to their potent anti-oxidative properties and ubiquity in various plant-based foods consumed (Cartea et al., 2011). The polyphenolic content produced by plants could relate to antioxidant activity. The antioxidant activity consists of phenolic compounds with their redox properties, which play a vital role in absorption and defusing free radicals, quenching single and triplet O_2 (Politeo et al., 2007;

Benedec et al., 2012). To fulfil these requirements, the application of mineral elements is necessary. Reportedly, fertilisation influences the phytochemical quality of plants. The application of N fertiliser reduces the antioxidant levels and enhances the antioxidant content in plants (Ibrahim et al., 2013). Irrigation, storage duration, harvesting time, temperature during storage, light exposure, disease and insects are factors that might affect the phytochemical content of herbs (Bottcher et al., 2003). The post-harvest storage duration (shelf life) of fresh herbs can have a substantially affect at pre-harvest. Incorrect plant density and N fertiliser application on herbs and leafy greens can reduce the growth, yield, quality, and phytochemicals on herbs. There is limited knowledge about the effect of N application, plant density on growth, post-harvest storage duration and phytochemical quality of basil and rocket.

Herbs yield was high with increasing dosages of N, although, increased N supply significantly reduced the accumulation of major phenolics, such as catechin, chlorogenic acid, hyperoside, quercitrin, isoquercitrin and TPC. Nitrogen deficiency causes many phytochemical and physiological disturbances leading to the reduction in cell division rates and perturbation in process of photosynthesis (Elhanafi et al., 2019). Frequently, there is a single-density plant adopted, which does not successfully yield high-quality perilla. It is crucial to formulate methods to improve the nutritional quality of herbs (Callan et al., 2007). Optimum plant spacing will improve yield and quality, including the reduction of input cost of seeds and amount of fertiliser without reducing yield. Phytochemical content in plants depend on several factors, including heredities, shelf life (Li et al., 2012) and agronomic practices (plant density and N fertilisation). Changes in the composition and content of the bioactive compound in food are likely to have an impact on the bioavailability and biological activity (Li et al., 2012).

Phytochemicals are secondary metabolites produced and used by plants as for protection against natural enemies; they are a key component for preventing human diseases and maintaining good health (Li et al., 2012). Consumption of fresh plant-based products high in phytochemicals and antioxidants has can help people overcome their degenerative conditions (Alothman et al., 2009). Unsuitable handling, processing and extended storage can reduce the healthy compounds available in produce (Alothman et al., 2009). Growers need to understand the importance of optimisation of the N fertilisation to avoid the negative effect of high fertilisation on the composition and phytochemical quality of plants. It is important to determine the effect of pre- and postharvest on the yield of leafy vegetables and phytochemicals content to improve the economic rate of the grower and maintain the health routine of consumers. The study was undertaken to explore the influence of N application rate and planting density on yield, phytochemical quality, antioxidant capacity and storage of basil and rocket. The aim of this study was to evaluate the hypothesis that N fertiliser and plant density would affect the yield and content of phytochemical quality of basil and rocket. There have been few studies conducted on the effect of N application rate at different planting densities on phytochemical quality and antioxidant level of herbs.

1.3 Objectives of study

- Investigate the influence of N application and plant density on growth and the yield of basil (*Ocimum basilicum* L.), and rocket (*Diplotaxis tenuifolia* (L.) DC.)

- To determine the effect of N fertiliser application and plant density on leaf mineral content of basil and rocket leaf content
- To determine the influence of N fertiliser application and plant density on phytochemicals properties (flavonoids, phenols, and antioxidants activity) of rocket and basil

Condensing the above objectives into two manuscripts avoided salami type of publications.

1.4 Significance of study

There is a poor understanding of the relationship between cultivation practice and post-harvest storage. Plant spacing and fertiliser application are cultural practices that cause variations in post-harvest behaviour within herb species; these factors can also influence the yield and quality of culinary herbs and leafy greens (Simon et al., 1989; Lopresti & Tomkins, 1997). The requirement for satisfying the local market with lengthy post-harvest storage of herbs needs to improve the agronomic practice. The amount of plants per unit area is vital among the yield components to optimise yield (Maboko & Du Plooy, 2013). The demand for herbs is increasing in the South African market. Due to their healing properties, it is possible to use rocket and basil as medicinal plants and culinary herbs. The adequate spacing and N levels have consented to improve marketable yield and increase the quality standard of herbs. The study was undertaken to ascertain the effect of N, plant density and storage duration conditions on phytochemicals quality parameters concerning the nutrients content, growth, and yield of fresh basil and rocket crops.

Poor management of plant density and nitrogen fertiliser application on basil and rocket can reduce the growth, yield, quality, nutrients, oil content and phytochemicals on high value crops. There is little knowledge about the effect of nitrogen application and plant density on the growth, yield and post-harvest phytochemical quality of basil and rocket, and little work done regarding improvement in production technologies of herbs. There have been various studies conducted on the growth parameters of basil, and plant nutrition is one of the most important factors that increase plant productivity (Patel & Kushwaha, 2013).

Nitrogen fertilisation has previously shown to correlate directly with the growth, and yield of herbs (Sifola & Barbieri, 2006); however, the effect of nitrogen availability on the polyphenolic composition and antioxidants properties of basil has not yet been determined (Nguyen & Niemeyer, 2008). One of the challenges in controlling nitrate levels in produce, for growers and food safety authorities is the fact that there is appreciable variability in nitrate concentrations within species (Weightman & Hudson, 2013). Regardless of efforts over the years to advance the knowledge of chemical composition, these plant-based compounds still lack a detailed phenolic profile (Vallverdu-Queralt, 2015). The study aims to determine the suitable N fertiliser level, plant density, and post-harvest storage duration to improve the yield, leaf nutrient content, phytochemical content (TPC and TFC) and antioxidant properties of basil and rocket leaves.

1.5 Delimitation and scope of study

The current study focuses on the literature review on herbs (basil and rocket) influenced by N application rate and plant density on storage and phytochemical compounds. The data was collected in the open field and laboratory and analysed with the analysis of variance (ANOVA) using GenStat®, ver. 11.1 (Payne et al., 2008).

1.6 Structure of the thesis

This study consists of five chapters. The first chapter consists of an introduction and background to the study and research aspects. The second chapter provides the literature review of the study, the origins, health benefits, and phytochemical contents of basil and rocket. Chapter 3 discusses the improvement of yield and mineral content of basil and rocket as affected by plant density and N fertiliser application. The fourth chapter discusses the effect of N fertilisation application rate, plant density, and post-harvest storage on basil antioxidants activity, flavonoids, and phenols content. There were four experiments on growth, yield, and phytochemical quality of basil and rocket conducted separately, however, these were pulled together to make two experimental chapters. The connection of the rationale of the study, as a follow up from the thesis, appears in Chapter 3, the first article, and Chapter 4 indicates the second article. Chapter 5 discusses and concludes on N fertiliser application and plant density on the phytochemical content of the rocket and basil on post-harvest quality.

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

The identification of herbs is as leaves of a plant, and spices are dried parts, such as bark, berries, sprouts, seeds and roots (Guldiken et al., 2018). The terms ‘herbs’ and ‘spices’ could differ according to plant parts used, and the differing ways they are used. Since ancient times, the addition of culinary herbs and spices to food was to improve organoleptic properties and enhance flavour. Culinary herbs have also found use as preservatives in food and medicine (Yashin, et al., 2017), and are widely regarded as having medicinal properties (DAFF, 2017). Extensive studies have revealed the beneficial effects of certain herbs and spices on human health due to their high antioxidant activity (Yashin et al., 2017). In South Africa, there is a growing demand for healthy consumption of fresh-cut herbs and leafy greens, for salad. Herbs, leafy greens, and spice usage is appreciated as completely natural ingredients (DAFF, 2017).

The market for herb products fresh and dry herbs, raw and processed herbal products are considerable, as the universal herbal supplement market reached approximately $107 billion in 2017 (Dou, 2017). Wyatt (2015) stated that herbs that have customer appeal start with good cultivation practices, handling and packaging. Getting quality fresh-cut herbs to the market has much to do with producing a healthy plant. Growing herbs can be highly profitable and there are many available. The huge sellers for the herb business are chives, parsley, rocket, basil and coriander (Wallin, 2019). Fresh herbs are gaining popularity in the South African market as they add flavour to food. In recent years, the increased consumer demand for fresh market basil and rocket has led to the more intensive field and greenhouse production (Homa et al., 2014; Hall et al., 2012b).

Considerable work has taken place on herbs and leafy greens phytochemical properties. The uses of herbs are essential for nutritional purposes; they contain nutrient elements, vitamin phytochemicals and healing properties. The conducting of the study was to determine the rate of different N applications and plant density to improve the production techniques to increase the yield of basil and rocket. The pre-harvest practice and post-harvest storage duration are important in preserving the quality of fresh-cut leaves and improving the phytochemical content of basil and rocket. The report on the required application rate of fertiliser in soil and plant density is lacking. With this concept, there has been much work done on medicinal properties and health benefits of basil and rocket, these an apprehension on yield, phytochemical content and post-harvest duration of fresh herbs influenced by fertiliser application.

2.2 Origin and description of basil

The basil species (*Ocimum basilicum* L.) belongs to the family of *Lamiaceae* consisting of shrubs, perennial and annual herbs originating from the tropical and subtropical regions of South America, Asia and Africa (Abd-el-azim et al., 2015). It is naturalised as a wild plant in temperate regions around the globe, including North America and

Northern Europe, distant from its native range (Yaniv et al., 1998). *Ocimum* genus consists of 150 species (Pushpangadan & Bradu, 1995) and within the *Ocimum basilicum* L. species, there are several varieties that differ in the content of chemical parameters and the general morphological texture and structure (Grayer et al., 1996; Stanojkovic-Sebic et al., 2017). Basil plants (Figure 2.1) have gained the title “king of the herbs” (Albuguerque, 1996; Makri & Kintzios, 2007), with many varieties considered annual crops (Lupton et al., 2017). Due to the increased interest in the use of basil as ornamental herbs, Morales and Simon (1997) began a selection and breeding programme to recognise new ornamental basil (Morales et al., 1993; Tansi & Nacar, 2000).

Figure 2.1 Basil seedlings.

The plants can be shrubby or herbaceous and vary in size from 50 to 60 cm tall (Meyers, 2003; Sharafzabeh & Alizadeh, 2011), depending on the species. The leaves can be smooth, hairy, curly and shiny, and green to blue or purple, and the flower colour ranges from white to purple to lavender (Meyers, 2003). Green and aromatic leaved varieties are mainly cultivated (Nurzynska-Wierdak, 2007). Basil flowers (pale-pink or violet buds) cluster in six to 10 element sets in pseudo-verticals at the shoot top forming pseudo spikes, and open for the whole day with higher intensity just before noon. Basil's flowers are open and insect-pollinated; having a short corolla tube, they are an easily accessible nectar source for insects that pollinate plants such as honeybee, solitary-bee and bumblebee (Bozek, 2000; Nurzynska-Wierdak, 2007). According to Lupton et al. (2017), basil's origin and growing conditions have an impact on the plant's uses, scent, medical uses and flavour. The essential oil content of basil has a similar variability amongst cultivars and species, believed to be the result of varying ecological factors, geographic origins, genetic patterns, different chemotypes and differences in the nutritional status of plants. Among *Ocimum* spp., the common basil is one of the economic species cultivated worldwide (Marotti et al., 1996).

2.3 Origin and description of the rocket

Rocket (*Eruca sativa*) belongs to the Brassicaceae family, endemic species mainly cultivated in Mediterranean regions such as Greece, Italy and Turkey. Rocket crops belong to two genera, *Eruca* and *Diplotaxis*, which are gaining popularity in the global market as a salad vegetable (Pasini et al., 2011; Bell et al., 2015; Bell & Wagstaff, 2019)., and in South Africa as a fresh-cut leafy vegetable and herb. The common names for rocket are rucola, arugula, or roquette variety (Martinez-Sanchez et al., 2006). The collection of rocket (*Eruca sativa*) used to be from the wild, but now it is cultivated domestically in gardens with other plants such as basil and parsley (Jafri, 1973; Marwat et al., 2016). Rocket leaves flavour salads and are characterised by a pungent taste.

Figure 2.2 Rocket seedlings.

The *Diplotaxis tenuifolia* (L.) DC is a diploid and perennial plant (Padulosi & Pignone, 1996), with dark green leaves, about 20 to 50 cm in height (Morales & Janick, 2002). The roots can subsist during winter and new sprouts develop in the spring. It starts flowering (2-4 cm) toward the end of spring to autumn and the seeds are ready for collecting during autumn. Rocket (Figure 2.2) adapts to severe and poor soils and can compete with other varieties in calcareous shallow soil (Padulosi & Pignone, 1996). This species has succulent leaves and mostly appreciated in cuisine and sold in small bunches in local markets (Padulosi & Pignone, 1996).

2.4 Health benefits of basil

Basil has uses in culinary, ritualistic and traditional medicine. In central India, the use of holy basil (tulsi) is for lifestyle rehearsal and spiritual rituals that provide health benefits (Singh et al., 2010: Lupton et al., 2017). The *Ocimum* spp is a flavourant for food, essential oil, fragrance and traditional medicines (Vieira & Simon, 2005). Basil is a good source of various essential oils, possesses delicacy and flavour as a spice, and fragrance and beauty as an ornamental (Carovic-Stamko et al., 2011). The utilised parts of the plants are seeds and leaves (Khatri et al., 1995). Basil is utilised to produce homeopathic medication to treat several diseases. The *Basilici aetheroleum* essential oil has uses in perfumes, sustenance and cosmetics industry to process insect repellent, fungicidal, bactericidal, anti-diarrhoeal, antiviral and radio-protective activity (Stanojkovic-Sebic et al., 2017). Hot basil tea treats nausea, dysentery and flatulence. Basil formulation treats skin infections, acne, insect stings and snakebites. Basil is used for treating several conditions such as insanity, convulsion, cancer, deafness, whooping cough, diarrhoea, epilepsy, toothaches and sore throat (Khatri et al., 1995).

Basil is utilised for food preservation (Suppakul et al., 2003), and basil volatile oils are exhibited to contain biologically active constituents for plants pathogens that are insecticidal, nematocidal, fungistatic and antimicrobial properties (Simon et al., 1999; Vieira & Simon, 2005). Dental and oral products also utilise the essential oil extracts of basil. Basil is an important component of many alcoholic beverages, including bitters, liquors and spirits, and a blended mixture of essential oils of fennel, basil, and coriander to a salt solution of whey, was reportedly a method to enhance the storage of a carbonated fermented milk beverage (Lupton et al., 2017).

2.4.1 Medicinal properties of basil

Many culinary and medicinal herbs are of specific interest and used for the creation of raw ingredients consisting of phytochemicals with substantial antioxidant capacities and health benefits (Exarchou et al., 2002; Raimondi et al., 2006). Some species of basil have antimicrobial and antifungal properties; *Ocimum basilicum* and *Ocimum gratissimum* are described to have antibacterial, antimicrobial and the *Ocimum* x*citiodorum* ‘Citriodorum’ and *Ocimum kilimandscharicum* oils are antifungal (Van Oosteron et al*.,* 2001).

People do not think of basil as a medicinal plant, yet it has been utilised as traditional medicine by nations worldwide and has the potential for a variety of medical conditions. Sudan and India utilise the *Ocimum americanum* for skin parasites, and in Brazil, for kidney problems and rheumatism (Ernest, 1997). In Africa, the edible parts of the leaves and roots of *Ocimum* spp are for treating influenza and stomach cramps (Darrah, 1980). The Chinese use *Ocimum basilicum* for gum ulcers medicine, and haemostyptic during childbirth (Awang, 2000), and Indians are treating rheumatoid arthritis, anorexia, earache, menstrual irregularities and malaria with basil (Tucker & Mayer, 2003).

Chemical components isolated from the plant, include alkaloids, tannins, terpenoids, saponin glycosides, and ascorbic acid. Basil consists of immunomodulatory, hepatoprotective, hypolipidaemic, antihyperglycaemic, antitoxic and antifungal properties (Khair-ul-Bariyah, 2012). Polyphenols’ biological active compounds are purposeful for the preparation of dietary supplements, nutraceuticals and functional food ingredients. Basil is utilised as an herb to treat kidney malfunction, headaches, coughs, constipation, gastrointestinal problems and warts (Caleja et al., 2017).

The establishing of aroma compounds might be in chemotypes of basil known as methyl chavicol, eugenol, linalool, and methyl cinnamate, and imported to the global essential oil market (Simon et al., 1999). The basil essential oil has curative properties and substances revealing antibacterial properties consisting of phenolic and flavonoids compound (Nurzynska-Wierdak, 2012). Antioxidants are an important part of maintaining a healthy and balanced lifestyle, and basil is an important source of these essential compounds (Tilebeni, 2011; Lupton et al., 2007).

2.5 Health benefits of rocket

Rocket is utilised for various purposes such as food, cosmetic and medicinal treatment, depurative, stimulant, and gastral activities (Cavaiuolo & Ferrante, 2014). It is used for increasing sperm production, fertility and treats eye infection (antibacterial), is helpful in the digestive process and kidney activities and is responsible for different pharmacological and biological activities, such as anti-fungal and antimicrobial (Marwat et al., 2016). Rocket leaves contain low calories when consumed raw as garnish and added to leafy salads; cooking rocket leaves will result in loss of nutrients and lessening of healthy compounds (Cavaiuolo & Ferrente, 2014). Rocket leaves have properties such as astringent, digestive, diuretic, emollient, gastric, depurative, laxative, rubefacient, a tonic for colitis and stimulant properties (Yaniv et al., 1998; Barlas et al., 2011).

2.5.1 Medicinal properties of rocket

Rocket species is a rich source of phytochemicals, carotenoids, vitamins, fibres, minerals, isothiocyanates, glucosinolates, and flavonoids such as flavonols, kaempferol, quercetin, isorhamnetin and phenolic compounds (Garg & Sharma et al., 2014). The rocket oil contains methyl-sulphinyl butyl, isothiocyanate and glucosinolate that induce enzyme activity anti-diarrhoeal, antibacterial, anti-tumour, analgesic, anti-diabetic, and anti-inflammatory activity (Marwat et al., 2016). Rocket is a good source of anti-cancer, antioxidants molecules and sulphur compounds. Phytochemistry analyses revealed a high content of health-promoting compounds in leaves and seeds, mainly antioxidants and glucosinolates (Cavaiuolo & Ferrante, 2014). Glucosinolates available in Brassicaceae consist of anti-carcinogenic, antifungal and antibacterial. Cruciferous crops act as substantial sources of antioxidants because of their high levels of carotenoids, tocopherols, and ascorbic acid (Cartea et al., 2011).

2.6 Economic importance of herbs basil and rocket

Culinary herbs and leafy greens production and processing is a growing industry. High-value minor crops are clustered into herbs and spices, aromatic and medicinal plants. From the evidence fact provided in 2006, each species contributed a separate output; the importance of these plants in total reached the global trade of US$ 60 billion (Sher, 2014). Medicinal plants and herbs are important in the economy of low-income states and remain critical and strategic because medicines are crucial to maintaining a healthy living that propels and keeps the economy balanced. In the survey conducted in an area around Los Angeles, basil was the most popular crop among

500 restaurants; of all types of restaurants, 7970 of them used basil (Putievsky & Galambosi, 1999). *Diplotaxis tenuifolia* (L.) DC., and *Eruca sativa* Mill gained popularity as baby leaf crops; consumers’ demand for them is due to their convenience, nutrition, and easily available product. There are similarities between an annual garden rocket and perennial wall rocket cultivars; propagated to look comparable, and supplied throughout the year (Hewett, 2006). Basil is economically important, with the essential oil used in sanitation, cleaning, perfumes, cosmetics, anaesthetic and antiseptic products (Liber et al., 2011).

Basil has documented high market returns in 2007 and 2013, with 80 tons sold in the fresh produce markets in South Africa. In 2006, the basil price eased higher despite an 11% rise in capacity supplied at the market (DAFF, 2017). Rocket production is for commercialisation around the globe and procured in supermarkets and farmers' markets (Yanivn et al., 1998). The information on annual market standards and sales volume is limited to basil and rocket plants (Jafri, 1973; Marwat et al., 2016).

2.7 Nutritional values in basil and rocket leaves

Herbal plants constitute valuable raw material, the biological activity of which depends on the content of the major biologically active substances and accompanying macro- and micronutrients, (Table 2.1) enzymes, and vitamins (Nurzynska-Wierdak, 2012). Minerals are important in body function; micronutrients constitute the active centre of enzymes and vitamins (Marschner, 1995). The mineral content Zn will help to decrease blood sugar levels and improve cholesterol levels, K helps to maintain healthy blood pressure (Ishola et al., 2017), and Fe is a component of proteins and is important for the transportation of oxygen (O), from the lungs to all body cells (IOM, 2000). The mineral content Mn plays a significant role in several physiological processes and is an activator of enzymes (Ursel, 2001). The availability of micronutrients in green plants supports the synthesis of organic compounds in plant tissues (Taiz & Zeiger, 2006) and acts as an enzyme activator in the human body (Fraga, 2005).

Basil is a green herb and provides several minerals in human nutrition (Leal et al., 2008; Javari & Asadi-Gharneh, 2017). It has a low calorific value, and contains carotene, vitamins A, B6, and C, as well as Ca, K, P, Mg, and Fe. The basil herb also contains flavonoids and is an antioxidant (Leonard et al., 2001; Dzida, 2010). The mineral contents, Ca, P, Mg, and S are the major building material for bones, teeth, skin, and hair, and play an important physiological function in the human organism. In addition to dairy products, raw plants are a source of these mineral components (Friedrich, 2002). Rocket leaves consist of nutritional and health-promoting compounds. Being a valuable source of carbohydrates and mineral nutrients (Table 2.2), rocket leaves have a high nutritional value (Nurzynska-Wierdak et al., 2012). Rocket is rich in vitamin K, which regulates blood clotting heals injuries and sores (Moynihan, 2020), containing 109 mg of the vitamin per 100 grams. Rocket is also a source of Vitamin C and Fe (Amorim et al., 2007).

Table 2.1 Nutritional value of basil per 100 grams (3.5 ounces), USDA National Nutrient Database, 2019 (Nordqvist & Gill, 2018).

Principle	Nutrient Value	Percentage of DV
Carbohydrates	2.65 g	1%
Protein	2.58 g	6%
Total Fat	0.64g	1%
Cholesterol	0 mg	0%
Dietary Fibre	1.6 g	6%
Energy	94 kilojoules (kJ)	
Vitamins		
Folate	60.00 mcg	
Niacin	0.902 mg	6%
Pantothenic acid	0.209 mg	4%
Riboflavin	0.076 mg	6%
Thiamin	0.034 mg	3%
Vitamin C	18 mg	20%
Vitamin A	5275 IU	106%
Vitamin E	0.80 mg	4%
Vitamin K	414.8 mcg	9346%
Electrolytes		
Sodium	4 mg	0%
Potassium	295 mg	6%
Minerals		
Calcium	177 mg	14%
Copper	0.386 mg	43%
Iron	3.17 mg	18%
Magnesium	64 mg	16%
Manganese	1.148 mg	50%
Phosphorus	56 mg	8%
Selenium	0.3 mcg	1%
Zinc	0.81 mg	7%
Phyto-nutrients		
Carotene-ß	3142 mcg	
Carotene-α	0 mcg	
Lutein-zeaxanthin	5650.00 mcg	

Table 2.2 Arugula rocket raw, Nutrition value per 100 g. ORAC value 1904. USDA, National Nutrient Database, 2019.

Principle	Nutrient Value	Percentage of DV
Carbohydrates	3.65 g	1%
Protein	2.58 g	5%
Total Fat	0.66 g	1%
Cholesterol	0 mg	0%
Dietary Fibre	1.6 g	6%
Energy	105 kJ	
Vitamins		
Folate	97 mcg	
Niacin	0.305 mg	2%
Pantothenic acid	0.437 mg	9%
Riboflavin	0.086 mg	7%
Thiamin	0.044 mg	4%
Vitamin C	15 mg	17%
Vitamin A	2373 IU	47%
Vitamin E	0.43 mg	2%
Vitamin K	108.6 mcg	90%
Electrolytes		
Sodium	27 mg	1%
Potassium	369 mg	8%
Minerals		
Calcium	160 mg	12%
Copper	0.076 mg	8%
Iron	1.46 mg	12%
Magnesium	47 mg	12%
Manganese	0.321 mg	14%
Phosphorus	52 mg	7%
Selenium	0.3 mcg	1%
Zinc	0.47 mg	4%
Phyto-nutrients		
Carotene-ß	1424 mcg	
Carotene-α	0 mcg	
Lutein-zeaxanthin	3555 mcg	

2.8 Factors affecting the growth of herbs

Environmental factors surrounding temperature, accessibility of water and quantity of water and daylight length, affect the growth rate of plants (Borges et al., 2018). Agronomic factors, amongst which the level of mineral nutrition is particularly evident, can adapt the content and chemical profile of several bio substances (Nurzynska-Wierdak, 2015). The recent information on phytochemicals in Brassicaceae are influenced by the climatic factor that can affect scientific and economical interest and can be the base to elaborate strategies for crop production, minimising usage of agrochemicals, planting plagues and disease resistant plants and improving the productivity with a high nutraceutical potential (Borges et al., 2018). High temperatures are the cause of isomerisation processes and reduced food nutritional values (Colle et al., 2016). The thermal regime around the roots can affect the absorption of water, nutrients, roots, and shoots growth (Yan et al., 2012; Schmidt et al., 2017). The expression of the productive potential of the rocket salad depends on the environment, varieties choice is also decisive for the success of the cultivation system adopted (Echer et al., 2001).

2.8.1 Nutrient elements required for plants growth

For sustainable crops production in agronomy, there has to be key nutrients applied to recompense those elements and removed from the cultivation system through exportation to the environment (Pretty & Bharucha, 20014). Planned fertiliser application to increase marketable yield is a significant objective in the agricultural system supplementing the requirement of the crops for nutrients through fertilisers.

Low nutrients availability is a serious constriction on food production (Kanwal et al., 2016). The nutritional condition of plants affects yield and quality as well as plant resistance to stress factors. Plants require 17 essential elements for growth and development, including hydrogen (H), carbon (C), oxygen (O), nitrogen (N), phosphorus (P), potassium (K), sulphur (S), calcium (Ca), magnesium (Mg), boron (B), chlorine (Cl), copper (Cu), iron (Fe), manganese (Mn), molybdenum (Mo), nickel (Ni), and zinc (Zn) on soil organic matter, inorganic and fertilisers (Uchida, 2000). The first group is the three macronutrients that plants can obtain from the air, water, carbon (C), oxygen (O) and hydrogen (H) (Nahler, 2004). These macro- and micro-elements are those commonly supplied to plants in fertilisers. Deficiency in one of these mineral elements may result in a decrease in plant growth, crop yields, and soil fertility (White & Brown, 2010). An optimal supply of macro- and micronutrients to plants reduces production costs, on the proviso that light, thermal and moisture conditions are adequate, ensures high-quality yield, and restrains the risk of environmental contamination (Nurzynska-Wierdak, 2012).

Macronutrients play a substantial role in plant growth and development. An adequate supply of macronutrients increases the yield, growth and quality of crops (Tripathi, 2014). Plants need large amounts of N for adequate growth. Nitrogen is absorbed or taken up as NO_3- or NH_{4+} from the soil for plant growth. The N regularly affects the amino acid composition of protein and in turn its nutritional quality (Chun, 2017). The N is the nutrient element with the highest concentration in nutrient solutions for leafy vegetables (Cavarianni, 2019), and plays the role as a yield-stimulating nutrient and affects both volumes of yields and the chemical composition of yield components (Chen et al., 2004; Nurzynska-Wierdak, 2012). Nitrogen is deficient in plants if the older leaves turn yellow-green or yellow (Chlorosis); as the deficiency progresses the entire plant yellows. Phosphorus plays a vital role in Adenosine 5'-triphosphate (ATP) in the plants. ATP that forms through photosynthesis has P in its structure and progresses from seedling growth through to the establishment of grain and maturity, deficiency symptom; leaves turn dark green but have stunted growth (Nahler, 2004). The K plays a crucial role in plant water relations by regulating the osmotic potential influences in their photosynthesis, energy production, lipid metabolism, water relations and protein synthesis (Wang, 2013). The K affects the growth, and basil yield quantity and quality (Rao et al., 2007; Nguyen et al., 2010). The signs of K deficiency appear on the outer margins of older leaves as yellow or brown spots (Nahler, 2004). Sulphur is important for the synthesis of coenzyme A, which is important for fatty acid biosynthesis and oxidation of intermediates of the citric acid cycle, and for ferredoxin oxidation, which is important in photosynthesis and biological N fixation (Havlin et al., 2005). Excessive watering can produce deficiencies and low levels of S in soil organic matter. Calcium is vital for cell elongation, cell division, and cell structure; Ca deficiency symptoms appear at the growing tips (Nahler, 2004). Magnesium is a component of chlorophyll. Mg deficiency in biomass and photosynthetic CO_2 assimilation in plants is an extensive obstacle affecting the productivity and quality of crops and forestry (Hauer-Jakli & Trankner, 2019).

Micronutrients (Fe, Mn, Zn, Cu, B, CL, and Mo) are required in a very small quantity, and contain less than 250 ppm or 0.025%, of dry plant tissue. Micronutrient deficiency is widespread; 30% of cultivated soils globally are deficient in iron and 50% of world cereal soils are zinc deficient (Kanwal et al., 2016). Boron (B) promotes cell development, translocation of sugars, and is important for growth regulators. B deficiency symptoms appear on the growing tip of the plant; the tip is often deformed and shows evidence of chlorosis (Nahler, 2004). CL is a turgor regulation, resisting diseases, and photosynthesis reaction; deficiency symptoms are wilting of young leaves and chlorosis. Cu is involved with photosynthesis and component of enzymes; the deficiency sign is leaf tip dieback, loss of turgor in young leaves, and chlorosis. Fe is essential for chlorophyll synthesis and photosynthesis, a component of the enzyme; deficiency is chlorosis or yellowing among the veins of small leaves. Mo is important in N fixation by legumes; deficiency symptoms are yellowing of young plants and chlorosis of older plants. Mn activities are enzymes, chloroplast production, and cofactor in many plants' reactions, yellowing between the veins of new leaves. Zn is important for plant hormone balance and auxin activity, and deficiency symptoms are stunted growth reduced internode length small leaves are smaller than normal. Mo is required by symbiotic nitrogen-fixing bacteria in legumes to fix atmospheric N; lack of Mo shows pale leaves with interveinal, marginal chlorosis, and necrosis (Kaiser et al., 2005). Herbs constitute valuable raw material, whose biological activity depends on the content of the major biologically active substances and accompanying macro- and micronutrients, enzymes, and vitamins (Nurzynska-Wierdak, 2012). Growing and yielding of basil similar to other cultivated plants, depends on the accessibility of all nutrients in the alimentary environment, moreover, the yield quality connects to the macro- and microelements taken up by the plant (Zheljazkov et al., 2008; Dzida, 2010). Agronomic practices, including fertiliser application, have not been standardised, and mineral necessities are unknown. Generally, lower yield is a warning aspect for these crops (basil, Swiss chard, and rocket) (Bulgari et al., 2017). Fertiliser application with an excessive or inadequate source of macro- and micro-nutrients may result in serious yield and quality losses. Poor fertilisation practices on soil or soilless media can result in nutritional disorders (deficiencies and toxicities) (FAO, 2017).

2.8.2 Light

Light is the most important environmental factor influencing the growth and development of a plant and herb quality, including secondary metabolism (phytochemicals content) (Guo et al., 2006; Dou et al., 2017). Light intensity levels can affect photosynthesis rates, tied to a plant's ability to grow (Guo, 2013). Bayat (2018) stated that light is the source of energy for growth and plant photosynthesis. The process of photosynthesis is sensitive to the factors of lighting environments. Light activates a varied range of signals and information for morphogenesis and physiological processes (Chen et al., 2004). Light aspects as spectral composition (wavelengths), intensity, length and direction can influence plant growth and development. The quantity of light received during the day serves as an acclimatisation signal, causing plants to amend their morphology and physiology to maximise photosynthetic carbon gain as well as a signal to rise light tolerance and lessen photo-oxidative stress (Niinemets, 2010).

The plants have their optimum light intensity ranges for growth. Light intensity that is too low or too high influences photosynthetic physiology, morphology, and secondary metabolite production. Most plant species could develop morphological, anatomical, physiological, and biochemical modifications in response to dissimilar light intensities (Zervoudakis et al., 2012). Light intensity has an impact on nutrient composition, growth, and dry matter accumulation of cucumber, pepper, and purslane (Wu et al., 2008; Ding et al., 2010). Plant growth depends on a series of exchanges that include the existence of light; in bayberry trees in high light conditions, there was a delay in growth due to light irradiance reducing photosynthetic rates (Guo et al., 2006). Low light decreases the biomass of leaves, stems, roots, and crops, as well as the transpiration, photosynthetic rate, and stomatal conductance of water vapour (Zhang et al., 2003; Zervoudakis et al., 2012). An increasing growth rate influenced by red light is associated with an alteration in the morphology of the crops (Miller & Machlis, 1968). In the instance where bluer light hits the plant, the more the light moves towards the ultraviolet range, the more energy hits the plant; this indicates herbs grown outdoors, have a stronger taste (Ottosen, 2018). Herbs grown in wideband sunlight that shifted in intensity and spectral distribution ensued in diverse yield and phytochemical concentrations (Dou, 2017).

2.8.3 Temperature

Temperature is the environmental aspect that regulates plant growth, development, and metabolism (Kaufman et al., 1999). The climatic factor, temperature, can reduce the production of determinate plants in equatorial and tropical regions (Ruelland & Zachowski, 2010; Borges et al., 2018). Climate change causes warm temperatures that have the potential to influence plant productivity (Hatfield & Prueger, 2015). Most tropical or subtropical plants are affected by low temperature, which can cause wilting, reduce fresh mass and the accumulation of roots dry matter, but such crops, to a lesser extent, are sensitive to moderate to high temperatures ranging from 15 and 32ºC (Basra, 2001). Warmer temperatures affect the reproductive stage of the development of plants (Hatfield & Prueger, 2015). Temperature stress can cause changes in the plant's chemical constitution (Borges et al., 2018). Basil requires warm temperate conditions and is best cultivated in subtropical and temperate regions; the optimum temperature for the germination of basil plant is 20ºC, with minimum and maximum temperatures of 7 to 27ºC, respectively. Basil plants are prone to frost and cold conditions and develop best in long day, full-sun (DAFF, 2012) and warm conditions (Lopresti & Tomkins, 1997). Basil germinates well over a wide range of temperatures, and the height of plants increased in day-time temperatures ranging from 21ºC and 30ºC, and the maximum yield of dry matter was reached at 30ºC (Chang, 2005). Environmental factors such as light, temperature and relative humidity are affecting germination (FAO, 2017). The plant’s reaction to the temperature is dependent on the variety and growing conditions (Lawlor, 1993).

Temperate crops are susceptible to heat stress when grown at high temperatures (above 30ºC), which can cause poor root development, mineral deficiency and limitation of photosynthesis. Heat-stress causes scorching of shoots, abscission and senescence of leaves, growth inhibition and reduced plant productivity (Giri et al., 2017). The production of rocket plants can occur throughout the year, but mild temperature conditions favour their growth. The low density of plant spacing during winter would have been a temperature effect (Filgueira, 2003), with the small seeds sensitive to low temperatures, below 20ºC. Seeds of the annual garden rocket are less sensitive to lower temperatures and there were no negative impacts seen at 10ºC (Hall et al., 2012b). Rocket has grown in temperatures ranging from 16-28ºC summer, 5-18ºC in winter, and 10-24ºC spring, with minimum and maximum temperatures respectively (Hall et al., 2012b). Temperatures above 30ºC can make leaves more rigid and spicier, and with a premature floral tassel, the plant may be anticipating the reproductive stage (Filgueira, 2003). The temperature should range from 17ºC at night and 25ºC during the day for better crop growth and development (Furlani et al., 1999; Schmidt et al., 2017). In temperatures ranging from 2 to 25ºC and an increase in day length, cool-season rocket crops grows faster (Hall et al., 2012b).

2.9 Factors that affect phytochemicals

Environmental factors including temperature, light, moisture availability and mineral nutrient, influence he post-harvest quality of leafy vegetables (Mattheis & Fellman, 1999; Gutierrez-Rodriguez, 2011). According to Borges (2018), environmental factors, such as salinity, climate and abiotic factors, promote phytochemical responses, causing a change in the quantity and quality of phytochemical compounds, such as polyphenol compounds, carotenoids, vitamins, glucosinolates, and polyamines. Environmental systems or factors, including high tunnel, UV, fertilisation, visible light and irrigation, influence bioactive compounds in vegetables. Genotypic factors determine the phytochemicals compound profile of fruits and vegetables, nevertheless, the phytochemicals compound contents and the biosynthesis activities have a strong influence from environmental factors, such as cultivation, irrigation light, fertilization, and post-harvest conditions (Xu et al., 2019).

The accumulation and synthesis of phytochemicals in crops depend on numerous aspects, such as post-harvest storage, genetics and processing conditions. Numerous pre- and post-harvest aspects, including cultivation practices, ecological factors (microclimate, soil type, location, and growing season) plant ripeness, processing, and post-harvest storage, influence the production of bioactive compounds in crops, and hereditary is the key factor amongst all. Industrial processing, including sterilising, freezing, blanching and canning in addition to several cooking procedures (steaming, boiling and microwaving), can modify the quantity and composition of nutrients with phytochemicals, resulting in minimised accessibility of these compounds. Processed products (crops) have less nutritional values than their respective commodities because of the loss of nutrients during processing (Li et al., 2012).

2.10 Impact of nitrogen fertilisation on basil and rocket

Nitrogen is one of the rudimentary nutrients utilised by plants to build numerous organic compounds, such as peptides, amino acids, enzymes, proteins, or nucleic acids (Liu, 2014). The N in biology is shared with C, H, O, and S to generate amino acids, which are the building blocks of proteins. Amino acid is utilised in composing protoplasm, the site for cell partition and plant growth and development. Subsequently, plants enzymes are made of proteins, and N is required for all of the enzymatic reactions in a plant (Uchida, 2000). The N is an essential element for the development and growth of the plant; excessive N fertiliser application leads to high nitrate (NO_3^-) concentration accumulating in the edible parts of these leafy vegetables. Utilising these crops can injure human health, thus it is important to develop an appropriate strategy for the agricultural application of N fertiliser (Liu, 2014).

The N is a significant macro-element for economic vegetable production and is especially required for prosperous cultivation when grown on soil with poor minerals.

The extra N reduces the overall risk associated with crop production. There should be N management strategies utilised on vegetable farms to reduce the N disoriented to the environment (Belec et al., 2001; Yoldas et al., 2008). Plants require N for the production of protein and non-protein compounds, and most important is the element has the fastest take up. It is involved in a most biochemical reactions that occur in living organisms (Nurzynska-Wierdak et al., 2011).

The application of N fertiliser increases the protein in grain crops and the quality and quantity of dry matter in leafy vegetables. Reduction in cell division courses stunted growth. N deficiency explains early maturity in some plants, which results in a substantial reduction in quality and yield (Uchida, 2000). The application of organic N fertiliser is in the form of composts, animal and green manure on plants. N fertiliser available in inorganic form is liquid, granular and slow-release formulations. Cautious irrigation planning and the quantity and period of N fertiliser applications are obligatory to avoid groundwater pollution. Monitoring the irrigation water can help conserve the fertiliser, water and protect the environment (Uchida, 2000).

Rocket is a hyper-accumulator of NO_3^-, considered for a long time the main factor that causes gastro-intestinal cancer (Cavaiuolo & Ferrente, 2014). The incremented amounts of N and K mostly contributed to the incrementation of fresh leaf mass and yield of the rocket (Nurzynska-Wierdak, 2009). Basil development and yield depend on the range of climatic, fertilisation and cultivation factors. Basil is a species that requires substantial fertilisation, and it responds well to N fertilisation application (Golcz et al., 2006; Nurzynska-Wierdak, 2012), which significantly improves the weight of basil leaves and affects the contents of chloroplast dyes and essential oil yield

(Arabaci & Bayram, 2004; Golcz et al., 2006). Nevertheless, there is limited information about the effects of agronomical practices on leaf chemical composition (Raimondi et al., 2006).

2.11 Plant density on yield and quality of herbs

Growers need to increase their production by adopting appropriate strategies and techniques that will lead to sufficient and reliable yields; it is essential to establish the best agronomic practices for cultivation and utilisation (Amaglo et al., 2007). Plant density is an important variable for achieving maximum yields and uniform vegetable maturity. Arabaci and Bayran (2004) planted sweet basil in three plant densities (20, 40 and 60 plants$\cdot$m^{-2}) and revealed the highest amount of dry matter, percentage and the yield of effective substances was in 20 plants$\cdot$m^{-2} (Khorshidi et al., 2009). The effect of different plant densities on yield of dry material of thyme (*Thymus vulgaris*) showed the higher yield of dry material obtained was with 15 cm densities of planting. Among the various factors that affect the growth, time of planting and proper spacing are important. Keeping the above in view, the study undertook to reveal the effect of planting time and row spacing on the growth and yield of *Mentha piperita* (Sharma & Kanjilal, 1999).

Plant density and nutrient solution composition are critical factors to increase yield, reduce pathogen incidence, enhance dry matter production, improve antioxidant activity, and reducing NO_3^- content (Chen et al*.,* 2004; Fravel & Larkin, 2002). The mobility of the plants to cover the space between the rows led to less planting density per unit area and resulted in lower yield in 60 and 75 cm row spacing (Singh & Nand, 1979). In the case of 30 cm row spacing, there was a high density of plants observed, which might have led to competition for space, light, inefficient utilisation of nutrients, etc., resulting in less plant growth characters and lower herbage yield. The higher herbage yield under 15 cm row spacing could be due to optimum plant population, proper utilisation of moisture and nutrients by the plants, which resulted in more leaf growth per plant leading to higher herbage yield (Vadiel et al., 1980; Randhawa et al., 1984; Sharma & Kanjilal, 1999). Increasing plant spacing reduces lateral branch growth and pod set on low-order branches so the number of pods per plant is lower, but not regularly adequate to reduce grain yield per unit area (French, 2004).

Optimal plant density is achievable by establishing appropriate distances between the rows as well as in the rows of plants (Turbin et al., 2014). Developing technologies that allow the rational use of land for food production is therefore necessary (Nascimento et al., 2018). Furthermore, wild rocket (*Diplotaxis tenuifolia* L.) marketable yields and economic returns improved by increasing plant density, as in other leafy vegetables (Bianco et al., 1998). For leafy medicinal and edible herbs, the total leaf area assumes an obvious relevance in determining total yield. The total leaf area of leafy medicinal and edible herbs assumes an obvious relevance in determining total yield. Several studies have recognised significant effects of genotype and plant density on leaf area (Badi, 2004; Van Oosteron et al*.,* 2001).

2.12 Post-harvest handling practice

Postharvest losses in nutritional quality, particularly vitamin C content, can be substantial and are enhanced by physical damage, extended storage duration, high temperatures, low relative humidity, and chilling injury of chilling-sensitive commodities (Kevers, 2007; Navarro, 2006). Reportedly, 40-50% of horticultural crops produced in developing countries are lost before consumption, mainly because of high rates of bruising, water loss, and subsequent decay during post-harvest handling (Kitinoja & Kader, 2002). The main causes of post-harvest loss include lack of temperature management, poor handling, poor packaging material, and lack of education about the need to maintain quality (Kitinoja et al., 2011). These factors influencing post-harvest quality are the amount of irrigation, fertiliser quantity, pest control, and growth regulators, which can affect the overall quality and suitability for storage by modifying physiology, chemical composition, and morphology (Bekele, 2018). If not controlled properly, they lead to post-harvest losses on a large scale (Ahmad & Siddiqui, 2015).

The shelf life of basil is relatively short compared to other herbs, such as rosemary, oregano, and thyme, and 30% losses during shipment are common. Fresh basil is extremely tender and easily damaged by rough handling and desiccation (Tavarini et al., 2015), and is susceptible to chilling injury, and should not be stored below 5°C for extended periods. Basil that has been damaged by cold (chilling injury) turns black and is rendered unsuitable for sale; store and ship fresh basil at 5-13°C and 95% RH (Hamasaki et al., 1994). There have been experimentations conducted on both

Diplotaxis and Eruca species to determine the effects of post-harvest storage conditions on respiration rates and chlorophyll content (Koukounaras et al., 2007). Both species of rocket have high respiration rates (Martinez-Sanchez et al., 2008) leading to rapidly impaired visual quality, such as stem browning, tissue yellowing and general decay (Koukounaras et al., 2007; Bell, 2016).

Storage of *Eryngium foetidum* L. at 10°C in LDPE (Low-density polyethylene) packaging was effective in extending the shelf life for two weeks compared to only four days under the traditional (ambient) handling (Clement & Sankat, 1996). Negi and Roy (2001) also reported that storage of dehydrated green vegetables of savoy beet (*Beta vulgaris* var *bengalensis*, cv. *Pusa Jyoti*) and amaranth (*Amaranthus tricolor* cv. *Pusa Kiran*) at low temperatures effectively reduced degradation of phytochemicals (β-carotene, ascorbic acid and chlorophyll and helped in reducing browning. They also showed that the level of phytochemical contents may increase or decrease depending on the storage environment (Ismawaty et al., 2015). It is of paramount importance for consumers, scientists, and industrialists to understand how low-temperature storage of food items affects their bioactive compounds and properties (Galani et al., 2017).

2.12.1 Storage

Leafy vegetables are extremely perishable, therefore after harvest they need utilising immediately. Temperature plays a significant role in their rapid deterioration, which attributes to different biological and environmental factors (Ambuko et al., 2017). After harvesting, vegetables continue to ripen, respire, and transpire, consequently, resulting in lower quality and quantity between harvests and ingesting (Garande et al., 2019). Correct post-harvest handling to maintain the quality at harvest is required (Ambuko et al., 2017). Adoption of appropriate production measures, such as packaging, pre-cooling, storage and transportation, can avoid post-harvest losses, and extend storage duration. Post-harvest processes, such as packaging of leafy vegetables and refrigerated storing, can contribute to reducing the losses when they are used together (Garande et al., 2019). During picking and post-harvest, wounds also harm the chemical physiognomies and physical quality of packed leafy greens. The production of most leafy vegetables is in the field, harvesting is with machines or instruments, and post-harvest storage is at a cool temperature of 4°C to maintain good quality (Mulaosmanovic et al., 2021).

Leafy greens such as basil, lettuce and rocket are highly perishable, for the reason that the high surface to mass ratio has a short period and high transpiration rate that results in colour loss and accelerating senescence (Marquez & Sinnecker, 2007). Cooling of leafy vegetables after harvesting is the most important factor to maintain the quality and increase of shelf life (Schmitz et al., 2019). The processing of leafy vegetables, such as rinsing, chopping, grading and packing, lead to many post-harvest disorders, which affect the appearance, internal and external quality of vegetables. Leafy vegetables must be attractive and have a pleasing appearance when stored in the market for wholesalers as well as customers (FAO, 2006).

Various factors determine green leafy vegetables, browning, and decline in quality, chlorophyll and carotenoids. The oxidative reactions of phenolic compounds through polyphenol oxidase that produce o-quinones to several polymerised products result in the browning of sliced surfaces that are aesthetically unpalatable. Leaf pigments catabolism has a strong link to storing conditions. Low temperature regularly slows down all leaf metabolisms conserving the quality (FAO, 2006). Farmers and traders involved in the vegetable business face a challenge when transporting and storing leafy greens to the market, as their shelf life is limited. There is number of procedures available to extend storage duration, such as evaporative cooling, cold rooms and packaging material (Robin et al., 2020).

Lettuce packed under cooling and accurate relative humidity directly after harvesting reduces water loss can maintain fresh mass, flavour, texture and appearance (Schmitz et al., 2019). Yellowing of leaves as the outcome of chlorophyll loss, loss of textural properties, wilting and decay from pathological breakdown are examples of quality and deterioration in harvested leafy greens. Transpiration water loss is one of the physiological processes that cause leafy greens to deteriorate; it causes shrivelling, wilting, and a loss of crispness, succulence and firmness, which are components of freshness. Leafy vegetables become unsaleable when they lose 3% of their original fresh weight (Ambuko et al., 2017). Leafy greens such as basil, lettuce and rocket are highly perishable, for the reason that the high surface to mass ratio has a short period and high transpiration rate, which results in colour loss and accelerating senescence (Marquez & Sinnecker, 2007).

Phenolics and anthocyanins are sensitive to environmental factors pH, light and temperature during storage (Ersus & Yurdagel, 2007; Kapcum & Uriyapongson, 2017). Post-harvest storage, genetics, environmental factors and processing conditions can affect the phytochemical accumulation and synthesis in crops, Changes in phytochemical amount and composition in food may influence their biological activity and bioavailability. Storage temperature, chemicals and atmospheric gas composition are key aspects that affect the quantity and quality of phytochemicals. Low temperatures of 1˚C to 4˚C can have a different impact on the various group of phytochemicals, which might not always result in increased antioxidant bioactive compounds (Li et al., 2012). Storage duration (shelf life) and storage temperature might affect the beneficial properties and phytochemical compound of basil and rocket.

2.13 Packaging of herbs

Packaging coordinates the system of preparing products for storage transport, retailing, distribution, and use (Singh & Kaur, 2019). Packaging is an approach of using materials to wrap or protect produce or products for preserving and presentation of product quality and freshness. Good packaging can prevent vegetables from physical damage, and carefully handling of the vegetables during, harvesting, classing, processing, carriage, loading and unloading can reduce the mechanical damage. Packaging is an important part of all food processing operation; the correct packaging consists of market appeal, coherence, relevance, distinctiveness, uniqueness (product shape and type) and protectiveness (Singh & Kaur, 2019). The primary goal of fresh produce merchandising is to deliver the product to the consumer at such a point in the ripening scale, that it will achieve perfect maturity at the time of eating (Lee & Chandra, 2018).

Packaging materials and packaging methods are important postharvest factors that determine the quality of lettuce, basil and rocket during storage. Packaging, not too tight and not too loose, can compromise the quality of leafy vegetables resulting in wounding or physical damage. Products that are available for packaging or wrapping of vegetables are micro or laser perforated bags that contain a modified or unmodified atmosphere to preserve; cling wrap, shrink film, Ziplock bags, anti-fog bag, anti-mist bag, perforated polypropylene (PPP) 1320-hole, Non-PPP, PPP-4-hole, and Anti-Fog-PP polyethylene film are used to prolong shelf life. The particular type of packaging used depends on the perishability and shape of the product. There are five main classifications of stem products, green vegetables, leafy vegetables, soft fruits and hard fruits (Matche, 2005). Incorrect packaging can accelerate spoilage. Packaging protects products (leafy vegetables) from excess moisture loss, contamination and damage (Lee & Chandra, 2018).

Leafy vegetables have poor storing potential after harvesting due to moisture loss. Low temperature reduces the respiration rate and senescence, as well as the growth of spoilage microorganisms (Lee & Chandra, 2018). Different categories of polymeric film packaging material might prevent the deterioration processes of crops quality throughout storage, e.g., water loss results in a decrease and shrinking of Bok Choy, and leafy lettuce colour changed, with lower fresh mass.

Utmost polymeric films have lower water vapour transmission rates than fresh products; excessive relative humidity can develop inside the packages, resulting in moisture condensation, microbial development and degradation (Lee & Chandra, 2018). High relative humidity in the packaging results in too much moisture barrier causing fast spoilage owing to microorganisms. Bags (plastic or polyethylene) are the common and favoured retail packs because of their low material and packaging cost, and can be made of paper, perforated polyethylene or polypropylene film, plastic or cotton nets (Matche, 2005). The main role of fresh produce merchandising is to provide the product to the consumers at a point of ripening scale, where it will be perfectly mature at the time of consumption (Lee & Chandra, 2018).

Modified Atmosphere Packaging (MAP) is an active procedure for changing the gaseous composition in a packet. It depends on the interaction between the respiration rate (RR) produce, and the transference of gases through the packing material, without any additional control imposed on the gas composition at the start (Caleb et al., 2013). Anti-Fog-PP treatment might provide improved post-harvest qualities laterally with a lengthy marketable shelf life of about two weeks during storage at market display temperature (Lee & Chandra, 2018). The packaging of leafy vegetables occurs under refrigerated temperatures to prolong their shelf life. When the packaging and storage temperature for vegetables are not optimal, there will be a compromise in the freshness of the vegetables, causing an increase in food waste (FRX & Teo, 2019). Storage duration, temperature, humidity and atmospheric conditions are optimised for specific crops within the logistics chain, but these factors are frequently designed to prevent optical degradation rather than phytochemical breakdown (Schouten et al., 2009). Preservation of post-harvest quality is through modified atmosphere-controlled packages with low O_2 and high CO_2 (Cavaiuolo & Ferrente, 2014). During the cold storage, the packaging reduced moisture loss and minimised the degradation of chlorophyll in all fresh green leafy vegetables (Souzan et al., 2007).

2.14 Phytochemicals in herbs

Phytochemicals (plant chemicals) are known as bioactive nutrients available in vegetables, fruits, grains, medicinal plants and foods that may contain desirable nutrients and health benefits that can minimise the risk of major chronic diseases (Liu, 2004). The phytochemicals that potentially provide health benefits are phenolic acid, lignans, flavonoids (Figure 2.1) carotenoids, isoflavonoids, flavanols, flavone, phytoestrogens, ascorbic acid, glucosinolates, phytostrerols, terpenoids, imonoids, anthocyanidins, which are available in plants and act as antioxidants (Thakur, 2020). Phytochemicals comprise compounds such as phytosterols salicylates, polyphenols, saponins, protease inhibitors, phytoestrogens monoterpenes, sulphides, lectins and terpenes (Webb, 2013). Bioactive compounds such as steroidal saponins, vitamins and organ sulphur compounds transpire constitutively, and under stress conditions, there could be a boost in their synthesis depending on the growth environment and the stressor (Forni et al., 2019). Phytochemicals are a source of natural antioxidant concentrations that supplement the requirements of the human body and could defend against free radical mutilation (Altemimi et al., 2017).

Antioxidants reduce and control oxidative mutilation in foods by preventing or inhibiting oxidation produced by reactive oxygen species (ROS), in due course increasing the storage duration and quality of nourishments (Altemimi et al., 2017). Phytochemistry considers the structural compositions of these metabolites, the biosynthetic pathways, functions, and mechanisms of action in the living systems as well as its medicinal, industrial, and commercial applications (Egbuna et al., 2020). Phytochemicals play a role in the protection of human health when their dietary intake is significant (Samrot et al., 2009). The phytochemicals impart flavour colour, and aroma into plants, and protection from infection and predators (Jimenez-Garcia et al., 2018). Processing crops, such as freezing, steaming, boiling, and drying, can decrease the levels or quality of phytochemicals available in a food product (Webb, 2013).

Phytochemicals have changed with respect to cancer risk, depending on age and genetic makeup of a person. Phytochemicals such as genistein resveratrol, curcumin, sulforaphane and quercetin may enhance the action of chemotherapeutic agents used to treat cancer (Webb, 2013). The health benefits of individual phytochemicals are unknown: the contradiction of several phytochemicals, the fibre contents, minerals and vitamins originating in food and the interaction of phytochemicals (Webb, 2013).

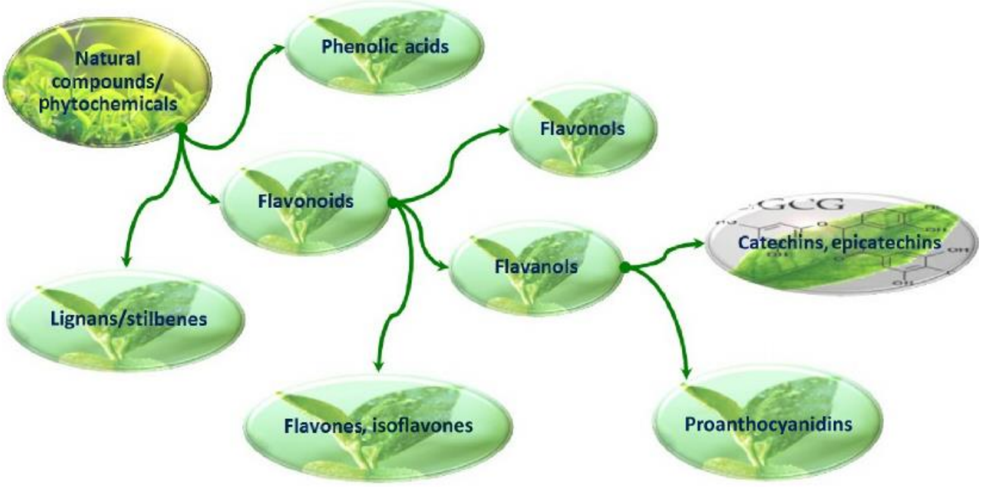

Figure 2.3 Classification of the principle type of phytochemicals in plants (Cojocneanu et al., 2015).

2.14.1 Natural antioxidants in herbs

Worldwide, there is a growing tendency toward the usage of aromatic, medicinal and leafy plants as antioxidants in diets (Yanishlieva et al., 2006). Primarily, the antioxidative effect of plants is due to the phenolic compounds they comprise, including flavonoids, phenolic acids and phenolic diterpenes, which could scavenge free radicals, donate hydrogen atoms or electrons, and chelate metal cations (Pietta et al., 1998; Kirka & Arslan, 2008; Amensour et al., 2009). Identified as sources of several phytochemicals, many herbs and spices possess powerful antioxidant activity (Dragland et al., 2003; Paur et al., 2011). Thus, herbs and spices may have a role in antioxidant defence and redox signalling (Paur et al., 2011).

An antioxidant is a substance capable of preventing or slowing the oxidation of other molecules. Normally, an antioxidant can protect against metal toxicity by trapping free radicals, thus terminating the chain reaction by chelating metal ions and preventing the reaction with reactive oxygen species (Flora, 2009). Antioxidant activity spices and herbs can treat some diseases (Yashin et al., 2017). Controlling antioxidants and reducing the oxidative damage in foods by delaying or inhibiting oxidation is caused by reactive oxygen species (ROS), ultimately increasing the shelf life and quality of these foods (Ames et al., 1993). Many agencies and healthcare systems worldwide recommend the increase in consumption of fruits and vegetables (Vivekananthan et al., 1993; Altemimi et al., 2017). Lately, polyphenols are gaining interest as antioxidants with the potential to reduce free radical-induced tissue injury. Flavonoids and phenolics are the active phenolic compounds isolated from higher plants

(Sankhalkar & Vernekar, 2016). Recently, people have become more health-conscious resulting in an increase in research on antioxidants (Mikami et al., 2009).

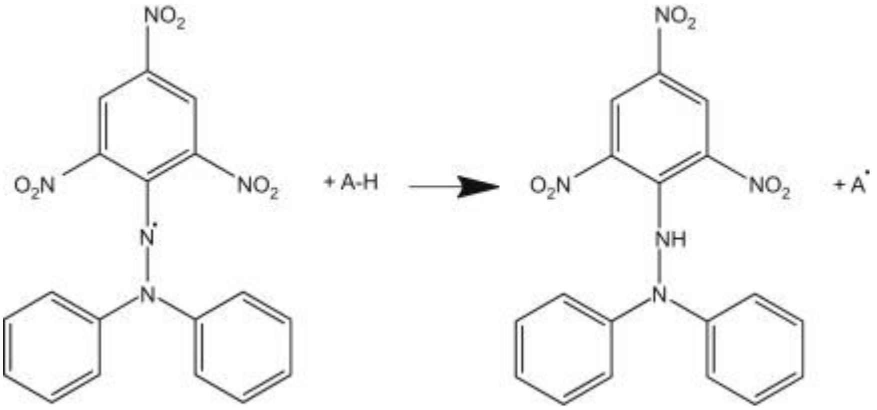

Figure 2.4 The reaction mechanism of DPPH radicals with antioxidants (Hernandez-Rodriquez et al, 2019; Campos, 2019).

The colour of this reaction changes from violet to pale yellow with the reduction of DPPH; the spectrophotometric method can determine antioxidant activity. The larger the antioxidant compound's capacity for free radical capture, the greater the reduction of DPPH and the less purple the sample retains. The expression of the results is as the effective concentration (EC_{50}), which corresponds to the amount of the sample necessary to decrease the initial concentration of DPPH radicals by 50%. This unit of expression allows comparison of results independent of the concentration of the sample (Carmona-Jiménez et al., 2014).

2.14.2 Beta-carotene

β-carotene is one of the secondary metabolites produced by plants and originates in an unoxidised compound group of carotenoids (Bogacz-Radomska & Harasym, 2018).

β-carotene is available in dark-green leafy vegetables and many orange and yellow coloured fruits and vegetables. β-carotene is the crucial precursor to retinol or vitamin A, and this conversion occurs in about 10% of the carotenoids (Olson, 1999; Giovannucci, 1999). The β-carotene occurs in the human diet and is a prominent member of the carotenoids group (Grune, 2010). The β-carotene compound obtained from a dietary supplement can lower the risk of cancer and heart diseases, before the conversion into vitamin A, and may account for much of the health benefits attributed to fruits and vegetables (Olson, 1999; Giovannucci, 1999). The conjugated polyene of carotenoids absorbs light or quenches free radicals during photosynthesis. The β-carotene can prevent erythropoietic protoporphyria and defend against carcinogens and mitochondrial DNA metamorphosis (Jia et al., 2017).

Dietary β-carotene was associated with probable reduced risk of oesophageal cancer but was unlikely to have a substantial effect on the risk of prostate and non-melanoma skin cancers (WCRFATCR, 2007). Excess of β-carotene in diets can accumulate the plasma carotene or cause hypercarotenaemia, in which the skin turns yellowish, especially on the soles of the foot and palms of the hand (NHMRC, 2006). β-carotene is a polyene compound, derivative from the $C_{40}H_{56}$ acyclic structure, and has an elongated chain of conjugated double bonds. High temperature isomerises double bonds that produce brightening of colour. A large quantity of β-carotene might be obtainable through chemical synthesis, but the original β-carotene is available from crops such as green leafy vegetables and carrots by physicochemical extraction. Phytochemical compounds of natural origin consist of high bio-accessibility and consumer acceptance in the market. Bioengineering is a great technique for β-carotene (Bogacz-Radomska & Harasym, 2018).

2.14.3 Phenolics

Phenolic compounds are secondary plant metabolics available in natural plant materials, including the plant kingdom (Giordano et al., 2017). The belief is that these compounds are an integral part of human and animal diets (Goncalves, 2017). They cover large and various groups of aroma compounds. The carbon atoms existing in the molecule categorise the type of phenolics compounds, and it is possible to classify the most common in plants as phenolic acids, flavonoids, tannins and tocopherols (Hannan et al., 2016). The production of phenolics is on a subepidermal layer of plant tissue exposed to pathogens and stress. The phenolic concentration in plant tissue depends on different growth, development stages and growing season (Bhattacharya et al., 2010).

The phenolic compounds gained interest during the last decade and contain antioxidant properties. Free radical-scavenging activities help in the prevention of oxidative stress conditions such as cancer, cardiovascular neurodegenerative and chronic diseases (Costa et al., 2021). Reportedly, phenolic and flavonoid compounds act as antioxidants to exert anti-allergic, anti-diabetic, anti-pathogenic, antiviral, anti-thrombotic, vasodilatory effects and heart problems, and Alzheimer-'s (Huyut et al., 2017). Phenolic compounds are obtainable from medicinal herbs and dietary plants including stilbenes, curcuminoids, coumarins, lignans, and quinones. Various bioactivities of phenolic compounds are responsible for their chemo-preventive properties (e.g., anti-carcinogenic, or anti-mutagenic and anti-inflammatory effects) and contribute to their inducing apoptosis by arresting the cell cycle (Huang et al., 2010).

Phenolic compounds play an essential role in their defence mechanisms, consisting of 8000 or more phenolic structures, varying from simple molecules, such as phenolic acids, to highly polymerised substances (Campos, 2019; Hernandez-Rodriguez et al., 2019). Phenolics cover a large group called phenylpropanoids, originating from phenylalanine, and have a six-carbon (C6) and three-carbon (C3) structure (Aldred, 2009). The basis for the classification utilised is the number of phenol rings and the structural elements connecting such rings, so that phenolic compounds are characterised as non-flavonoids and flavonoids (Costa et al., 2021).

2.14.4 Flavonoids

Flavonoids, known as polyphenolic secondary metabolites compounds with low molecular mass, commonly spread in green plants and situated in cell vacuoles. Flavonoids are a diverse group of biological activities available in plants, bacteria and animals. Flavonoids from plants are in synthesised positions and are responsible for scent and colour of flowers; they are abundant in green leaves, germination seed and spore, development and growth of seedlings (Samanta et al., 2011). The structural classification of flavonoids is as flavones, flavonols, flavanones, flavanonols, isoflavones, and flavanols (catechins). Anthocyanidins and chalcones, aurones, leucoanthocyanidins (flavan 3,4-diols), proanthocyanidins (tannin), and dihydrochalcones are contained in flavonoids (Terahara, 2015).

Flavonoids are the major class of plant polyphenols, with a common structure of diphenylpropanes, containing two aromatic rings connected to three carbons and abundant in medicinal plants and healthy diets (Qiu et al., 2018). The flavones are categorised by a planar structure due to double bonds in the central aromatic ring. (Nijveldt et al., 2001). Flavonoids include a group of compounds such as kaempferol, quercetin, catechins and anthocyanidins, possessing antioxidant activity and exhibiting a healthy benefit through the cell-signalling pathway (Nazni & Dharmaligam, 2014). According to Terahara (2015), flavonoids structures are categorised as flavanols (catechins), flavonols, flavonones, isoflavones and proanthocynanidinis (tanins). Researchers are interested in deciphering how flavonoids alter the functions of the human body, as consumption of flavonoids improves health (Qiu et al., 2018). Flavonoids are an important component for various nutraceutical, medicine, pharmaceuticals and cosmetics production (Panche et al., 2016). It has potential benefits properties in human health, such as antioxidant activities antiviral, anti-platelet, anti-tumour, anti-inflammatory and anti-allergy (Choudhary et al., 2013).

CHAPTER 3

Growth, yield and mineral content of basil and rocket due to plant density and nitrogen level

3.1 Abstract

There is limited information on how plant density and nitrogen (N) application rate for basil (*Ocimum basilicum* L.) and rocket [*Diplotaxis tenuifolia* (L.) DC.] affect crop growth, yield, and quality. A field trial took place to assess effects of plant density, N application rate, and their interaction, on growth and yield of basil and rocket during 2016 and 2017 growing seasons. The N levels (60, 90, 120, 150 or 180 kg·ha^{-1}) and plant densities for basil at 40,000, 62,500 and 93,750 plants·ha^{-1}, and plant densities for rocket at 40,000, 80,000 or 133,333 plants·ha^{-1} were used. Leaf chlorophyll content of basil improved with the application of N at 120 kg·ha^{-1}, while that of rocket improved at 120-180 kg·ha^{-1} N. Leaf yield of basil and rocket did not improve with N application at 60-180 kg·ha^{-1}. Fresh leaf mass and area increased with increased plant density for both crops, with higher yield in 2016 than 2017. Potassium leaf content of basil was highest at 150 kg·ha^{-1} of N and plant density of 40,000 plants·ha^{-1}. Plant density at 40,000 plants·ha^{-1} for rocket produced high K leaf content at 120 kg·ha^{-1} of N. Leaf N, Ca and Mg contents for basil improved at 62,500 or 93,750 plants·ha^{-1}. Application of 60 kg·ha^{-1} N at 93,750 plants·ha^{-1} for basil, and 60 kg·ha^{-1} of N at 133,333 plants ha^{-1} for rocket were suitable for improved leaf yield. Increasing plant density and providing

a reasonable amount of N could be useful to obtain high marketable leaf yield along with efficient N management to reduce environmental cost of production.

3.2 Introduction

Rocket [*Diplotaxis tenuifolia* (L.) DC.] and basil (*Ocimum basilicum* L.) are edible herbs reported to have medicinal properties (Mastaneh et al., 2017; Nurzynska- Wierdak et al., 2012; Simon et al., 1984). Plant density and nitrogen (N) levels are important factors in herb production (Arabaci & Bayram, 2004; Hall et al., 2012a). Whether plants compete for space, water, nutrients, or sun is largely dependent on how many plants are present per unit area (Sadeghi et al., 2009). Ideal plant density can lead to maximum yields; too high or too low plant densities can result in degraded yield and quality (Maboko & Du Plooy, 2015). Ideal plant density allows for ease of field operations and minimises competition among plants for light, water and nutrients, creating a favourable microclimate in the canopy to reduce risk from pests and disease (Maboko & Du Plooy, 2015; Sadeghi et al., 2009). There has been use of high N levels to improve crop yield, however, oversupply of N is not always beneficial, as it could result in reduced growth and yield of basil (Yassue et al., 2018), and the possibility of sub-soil water pollution. Insufficient N is associated with lower leaf chlorophyll content and lower yield (Yassue et al., 2018). Optimal N management improves crop yields and contributes to higher N use efficiency reducing environmental pollution (Sharma & Bali, 2018). It is important to understand the relationship between yield, planting density, and N application to balance agronomic and environmental objectives in a sustainable manner. This study aimed to evaluate N level and plant density on yield and leaf mineral content of basil and rocket.

3.3 Material and methods

The study was conducted from January to May in 2016 and 2017 (summer/autumn season) at the Agricultural Research Council - Vegetable and Ornamental Plants, Roodeplaat, Pretoria, South Africa: latitude of 25º35′ S, 28º21′ E, and 1,164 m above sea level. The average minimum and maximum temperatures were 13.7ºC and 38.2ºC, respectively. Basil, cv. Genovese (SeedCor, Pretoria, South Africa), and rocket seed (Hygrotech Pty. Limited, Pretoria, South Africa) were sown in 200 cavity polystyrene seedling trays filled with Hygromix® as a growing medium (Hygrotech Pty. Limited, Pretoria, South Africa) to produce transplants (Maboko & Du Plooy, 2015). The top 30 cm of soil at the site of a sandy clay loam consisted of 25% clay, 6% silt, and 69% sand, pH = 7.04. Chemical composition of the soil in 2016 was 29.5 mg·kg-^{1} phosphorus (P), 178 $mg \cdot kg^{-1}$ potassium (K), 1054 $mg \cdot kg^{-1}$ calcium (Ca), 0.021 $mg \cdot kg^{-1}$ N, 327 $mg \cdot kg^{-1}$ magnesium (Mg), and 54.3 $mg \cdot kg^{-1}$ sodium (Na). In 2017, the soil chemical composition was 1.1 $mg \cdot kg^{-1}$ P, 185 $mg \cdot kg^{-1}$ K, 927 $mg \cdot kg^{-1}$ Ca, 0.029 $mg \cdot kg^{-1}$ N, 281 $mg \cdot kg^{-1}$ Mg, and 50.6 $mg \cdot kg^{-1}$ Na. The arrangement of the trial was as a 6 × 3 factorial for both basil and rocket with N levels of 60, 90, 120, 150 or 180 $kg \cdot ha^{-1}$, and plant densities for basil at 40,000, 62,500 or 93,750 $plants \cdot ha^{-1}$ and for rocket 40,000, 80,000 or 133,333 $plants \cdot ha^{-1}$. Nitrogen application of 90 $kg \cdot ha^{-1}$ for both crops was the positive control, according to soil analysis recommendations (Anonymous, 2007). There was separate examination of each crop. Treatment combinations took place three times in a randomised complete block design. Soil preparation involved ploughing, disking and rotovating, with raised beds constructed. Phosphorus was broadcast as granular superphosphate (10.5%) at 20.5 $kg \cdot ha^{-1}$ in 2016 and 48.9 $kg \cdot ha^{-1}$ in 2017, according to soil analyses, and incorporated into the soil two days before transplanting. There was no potassium applied in both seasons, as it was sufficient according to soil analyses. Nitrogen was applied pre-plant (50% N) and top dressed at four (25% N) and eight (25% N) weeks after transplanting using limestone ammonium nitrate (28% N). The 2017 planting was in a similar soil type as in 2016 at different locations to the previous season, which probably accounts for differences in soil nutrient content. Five-week-old rocket seedlings were transplanted by hand on raised beds (20 cm high), 1 m wide and 3 m long (3 m^{-2} plot size); basil was transplanted on raised beds 1.2 m wide and 3 m long (3.2 m^{-2} plot size). Plants were watered with drip irrigation (three irrigation cycles per week) at a delivery rate of 2 $L \cdot h^{-1}$ and operating pressure of 150 kPa, which supplied 450 $L \cdot m^{-2}$ water throughout the season. Total rainfall during the growing season was 65.40 mm (2016) and 81.07 mm (2017). The control of weeds was by hand.

Four weeks after transplanting, five basil and five rocket plants were selected for leaf chlorophyll content measurements using a chlorophyll meter (SPAD) (Konica Minolta, Osaka, Japan), thereafter, measurements were taken every second week up to harvest. The first leaf harvest occurred at 35 days after transplanting (DAT) by cutting the plant 20 cm above the ground, with four consecutive harvests per crop. The harvesting of basil and rocket leaves took place bi-weekly. At harvest, fresh leaf mass, leaf area, dry leaf mass, and fresh and dry shoot mass were measured per plot. A leaf area meter (model LI-3100, Licor, Lincoln, NE) measured the leaf area. A convection oven (Thermal Products Solutions, New Columbia, PA) dried the plant leaves and shoots at 70ºC for 48 h for dry mass determination. After drying basil and rocket, the leaves were ground using a mill with a 1 mm sieve. The N content was determined using a Carlo Erba NA 1500 C/N/S Analyzer (Thermo Scientific, Waltham, MA)

(Jimenez & Ladha, 1993). An aliquot of the digest solution was used for Inductively Coupled Plasma Optical Emission Spectrometry for estimation of P, K, Ca and Mg concentrations (Olivier et al., 2012) on a dry mass basis.

The data underwent analysis of variance using GenStat® (ver. 11.1, VSN, Rothmsted, UK). In most instances, there was a quantitative, continuous component, and testing the data determined if they fit linear distributions. If the data did not fit linear distributions, and the interaction was significant, it was used to explain results. If interactions were not significant, Fishers' protected t-test least significant difference separated the means.

3.4 Results and discussion

Relationships between N application rate, plant density and fresh leaf mass, leaf area, leaf dry mass, fresh shoot mass, dry shoot mass, leaf chlorophyll and leaf mineral content for basil and rocket in 2016 and 2017 fit a linear model, and specific regressions differed in the level of significance and degree of association (Tables 3.1 and 3.2). N application during 2016 and 2017 affected leaf chlorophyll content of basil (Tables 3.1 and 3.3) and increased with an increase in N (Figure 3.1). Leaf chlorophyll content of basil in 2016 exhibited a moderately positive response with increasing N application. Leaf chlorophyll content of basil was highest at 120 kg·ha^{-1} N in 2017 with weak positive responses to N application rate (Figure 3.1, Table 3.1).

Table 3.1 Regression equations for leaf chlorophyll content and plant parameters of basil and rocket grown in two years and fertilised with 60-180 kg·ha^{-1} nitrogen.

Plant parameter	Regression equation	R2
Leaf chlorophyll (Y × N)[a]		
Basil (2016)	y = 0.0174x + 38.264	0.78
Basil (2017)	y = 0.029x + 36.038	0.23
Dry shoot mass (D × Y)		
Rocket (2016)	y = −0.0084x + 67.159	0.99
Rocket (2017)	y = 0.3848x + 516.76	0.99
Leaf mineral content (N)		
Rocket (N)	y = −0.0004x + 3.8974	0.026
Fresh Leaf mass (D × Y)		
Basil (2016)	y = 0.1032x + 4,560.30	0.95
Basil (2017)	y = 0.0357x + 7,154	0.80
Rocket (2016)	y = 0.1786x + 7,411.50	0.97
Rocket (2017)	y = 0.0935x + 7,030.90	0.92
Leaf area (D × Y)		
Rocket (2016)	y = 0.9287x + 42,258	0.97
Rocket (2017)	y = 0.5826x + 45,569	0.96
Leaf area (D)		
Basil	y = 513.53x + 3E+07	0.99
Dry leaf mass (D)		
Basil	y = 0.0056x + 561.30	0.97
Dry leaf mass (D × Y)		
Rocket (2016)	y = 0.0112x + 88,747	0.99
Rocket (2017)	y = 0.0068x + 1,155.80	0.69
Fresh shoot mass (D)		
Basil	y = 0.0246x + 2,123	0.98
Fresh shoot mass (D × Y)		
Rocket (2016)	y = 0.0032x + 190.05	0.97
Rocket (2017)	y = 0.0177x + 1,423.30	0.99
Dry shoot mass (N)		
Basil	y = 0.0046x + 302.31	0.99

Y, year; N, nitrogen

Table 3.2 Regression equations for leaf minerals of basil and rocket.

Leaf mineral	Regression equation	R2
Basil (N%)	y = 7E-06x + 4.2958	0.73
Basil (Ca%)	y = 2E-06x + 1.8493	0.65
Basil (Mg%)	y = 1E-06x + 0.6239	0.72

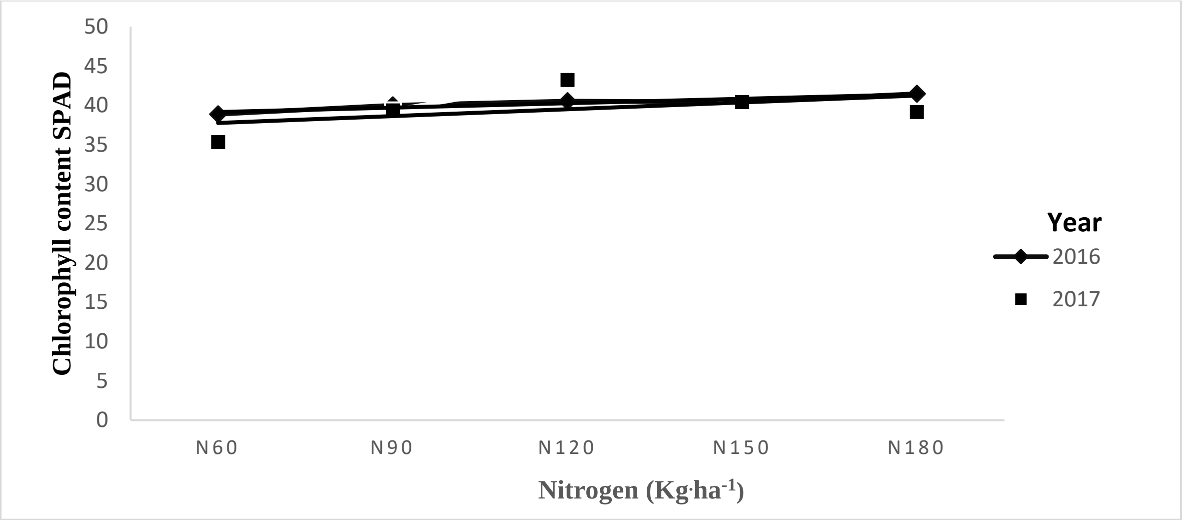

Figure 3.1 Interaction effect of nitrogen and year on leaf chlorophyll content on basil.

Table 3.3 Analysis of variance for the effect of nitrogen and spacing on leaf chlorophyll content in basil and rocket.

		Basil chlorophyll content, SPAD	Rocket chlorophyll content SPAD
Source of variation	df	MS	MS
Year (Y)	1	12.982^{ns}	427.629**
Residual	4	8.972	22.333
Nitrogen (N)	4	55.287***	6.294^{ns}
Plant density (D)	2	3.173^{ns}	8.796^{ns}
N × D	8	3.508^{ns}	7.068^{ns}
N × Y	4	25.601***	3.066^{ns}
D × Y	2	9.515^{ns}	6.124^{ns}
N × D × Y	8	1.884^{ns}	7.440^{ns}
Error	56	3.002	5.187
Total	89		

ns, **, ***not significant, or significant at 1% or 0.1%, respectively, ANOVA.
df, degrees of freedom; MS, mean square

Leaf chlorophyll content of rocket was higher in 2016 (avg. 48.16) than 2017 (avg. 43.80). which resulted in high fresh and dry leaf mass in 2016. Leaf chlorophyll content is a key factor affecting performance of plant photosynthesis (Taiz & Zeiger, 2006) associated with increased plant yield (Yassue et al., 2018). Nitrogen application improved leaf mass and increased leaf chlorophyll content, possibly due to effective use of photosynthetically active radiation (Taiz & Zeiger, 2006).

Table 3.4 Analysis of variance for the effect of nitrogen, year, and plant density on basil yield parameters.

Source of variation	df	Fresh leaf mass	Leaf area	Dry mass	Fresh shoot mass	Dry shoot mass
		MS	MS	MS	MS	MS
Year (Y)	1	7.478E+10**	2.171E+13***	1.333E+9**	1.266E+11**	5.075E+9**
Residual	4	2.633E+10	2.778E+10	8.146E+7	4.327E+9	7.159E+7
Nitrogen (N)	4	1.472E+9^{ns}	5.896E+10^{ns}	3.448E+7^{ns}	2.867E+7 ^{ns}	1.407E+7^{ns}
Plant density (D)	2	1.057E+11***	5.765E+12***	7.104E+8***	1.357E+10***	4.583E+8***
N × D	8	1.196E+9^{ns}	2.466E+11^{ns}	2.661E+7^{ns}	1.367E+8^{ns}	9539E+06^{ns}
N × Y	4	1.030E+9^{ns}	3.543E+10^{ns}	1.714E+7^{ns}	4.322E+8^{ns}	3.601E+06^{ns}
D × Y	2	3.436E+10***	7.651E+10^{ns}	7.714E+7^{ns}	8.551E+8^{ns}	3.965E+07^{ns}
N × D × Y	8	2.665E+9^{ns}	1.969E+11^{ns}	2.666E+7^{ns}	2.606E+8^{ns}	1.178E+07^{ns}
Error	56	2.085E+9	1.383E+11	3.931E+7	4.577E+8	1.391E+07
Total	89					

*, **, *** = significant at 5%, 1% ($p < 0.01$) or 0.1%, ANOVA.
df = Degrees of freedom, MS = Mean squares

In this field study, the application of N at 60 kg·ha^{-1} was sufficient to produce similar leaf yield of basil and rocket to increased N application rates of 90-180 kg·ha^{-1} of N. Nitrogen application rate did not affect basil and rocket leaf yield (Tables 3.4 and 3.5). Dry shoot mass of rocket was higher at 90-120 kg·ha^{-1} N in 2017 than N treatments in 2016 which were lower (Figure 3.2).

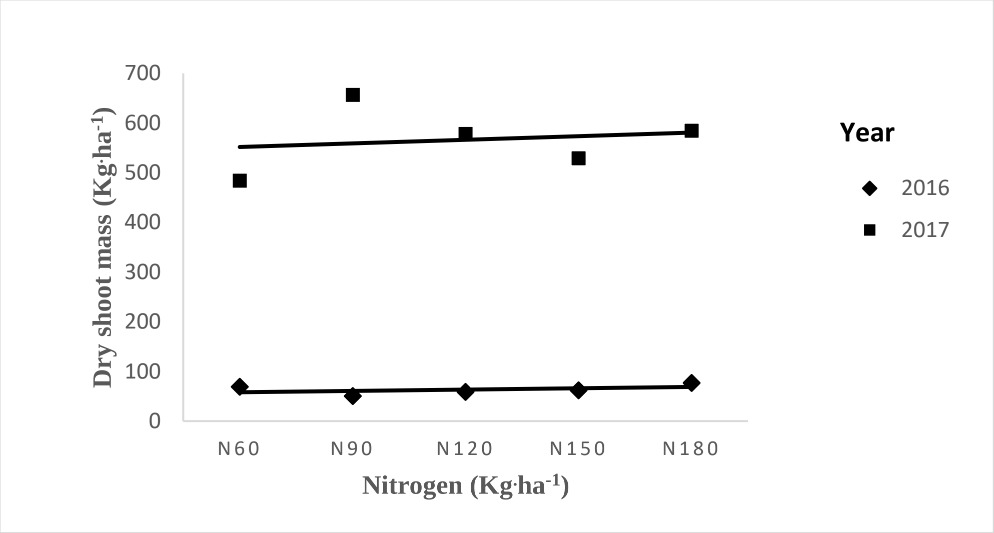

Figure 3.2 Effects of nitrogen rate and year on dry shoot mass of rocket.

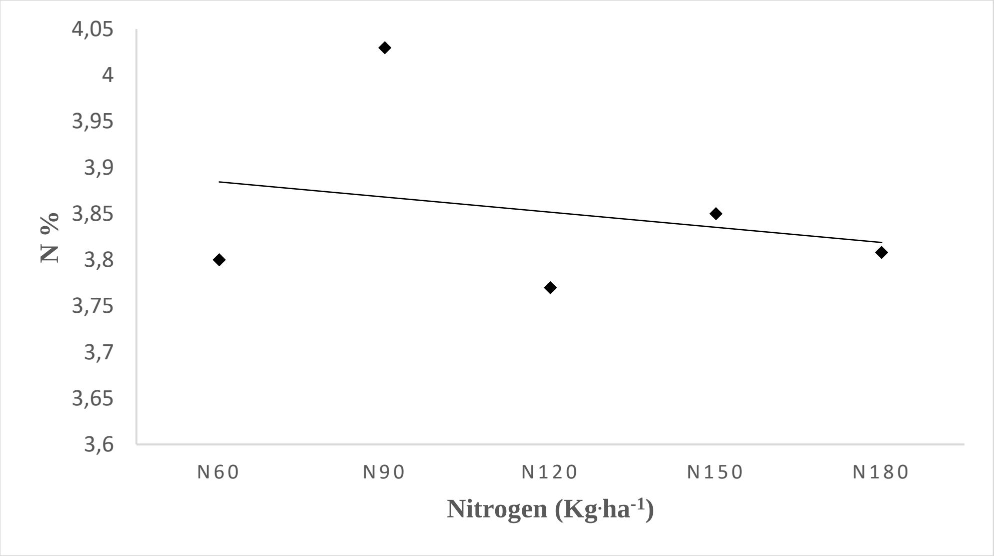

Figure 3.3 Effect of nitrogen on leaf nitrogen content of rocket.

There was a weak, relationship with N application on leaf N mineral content of the rocket (Figure 3.3). The highest leaf N content of the rocket was at 90 kg·ha^{-1}. Year influenced fresh leaf mass, leaf area, dry leaf mass, fresh shoot mass, and dry shoot mass of basil, which were higher in 2017 than 2016 (Tables 3.4 and 3.5). In rocket, there was greater fresh leaf mass and dry leaf mass in 2016 than in 2017; in 2017, leaf area, fresh shoot mass and dry shoot mass were higher than in 2016 (Tables 3.4 and 3.5).

Table 3.5 Analysis of variance for effect of year, nitrogen and spacing on rocket yield parameters.

Source of variation		Fresh leaf mass	Leaf area	Dry mass	Fresh shoot mass	Dry shoot mass
	Df	MS	MS	MS	MS	MS
Year (Y)	1	1.290E+12***	1.511E+13**	2.109E+08ns	1.363E+11***	5.680E+09***
Residual	4	1.372E+10	1.970E+11	4.497E+07	5.947E+08	2.841E+07
Nitrogen (N)	4	9.693E+09^{ns}	2.189E+10^{ns}	2.167E+07^{ns}	2.722E+08^{ns}	1.579E+07^{ns}
Plant density (D)	2	1.277E+12^{ns}	3.924E+13^{ns}	5.912E+09***	7.192E+09***	2.669E+08***
N × D	8	4.290E+09^{ns}	3.614E+11	8.416E+07^{ns}	2.651E+08^{ns}	6.860E+06
N × Y	4	2.371E+09^{ns}	1.205E+11^{ns}	6.710E+07^{ns}	2.776E+08^{ns}	2.199E+07*
D × Y	2	1.197E+11***	2.046E+12**	4.798E+08**	3.498E+09**	1.436E+08***
N × D × Y	8	5.169E+09^{ns}	8.624E+10^{ns}	3.201E+08^{ns}	3.209E+08^{ns}	1.030E+07
Error	56	6.454E+09	3.019E+11	1.159E+08	2.785E+08	8.034E+06
Total	89					

*, **, *** = significant at 5%, 1% ($p < 0.01$) or 0.1%, ANOVA
df = Degrees of freedom, MS = Mean squares

Differences in soil phosphorus content between seasons could be a reason for differences in leaf yields (Tables 3.4 and 3.5). At 62,500 and 93,750 plants·ha^{-1} of basil, K leaf content remained comparatively stable at various N rates, but differences occurred at 40,000 plants·ha^{-1} with an increase at 60 kg·ha^{-1} N, remaining stable until a decline at 180 kg·ha^{-1} (Figure 3.4). Potassium leaf content of the rocket was higher at 40,000 plants·ha^{-1} reaching maximum at 120 kg·ha^{-1}. Nitrogen ranging from 150 to 180 kg·ha^{-1} N at 80,000 plants·ha^{-1} had the lowest K leaf content (Figure 3.5).

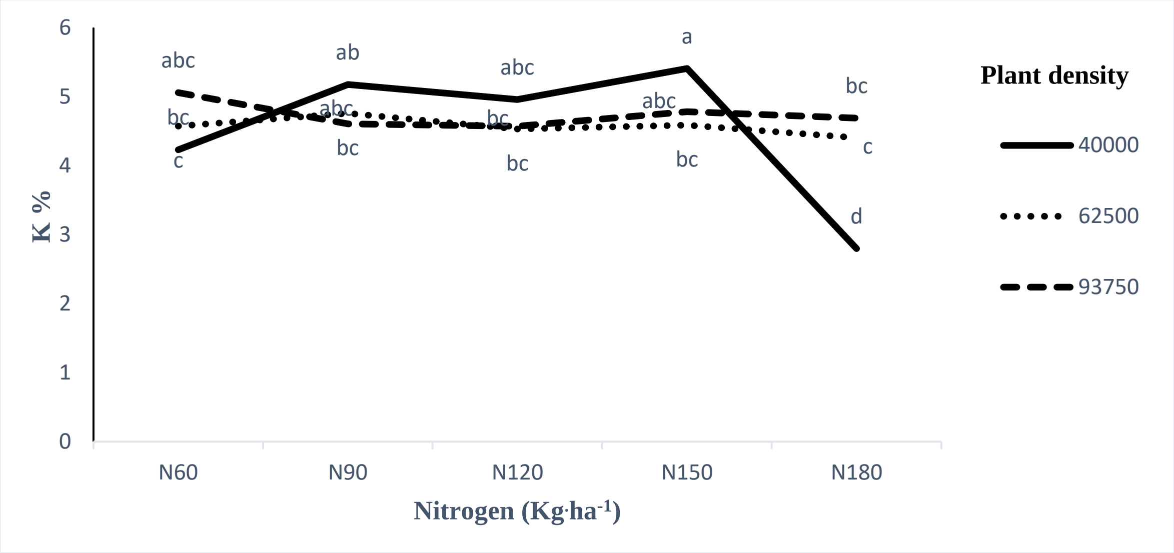

Figure 3.4 Interaction effect of nitrogen and plant density of leaf potassium (K) content of basil. Points on lines with the same letter are not significantly different. Data in the interaction were analysed with Least Squares Means and means separated at $p \leq 0.05$ with Least Significant differences.

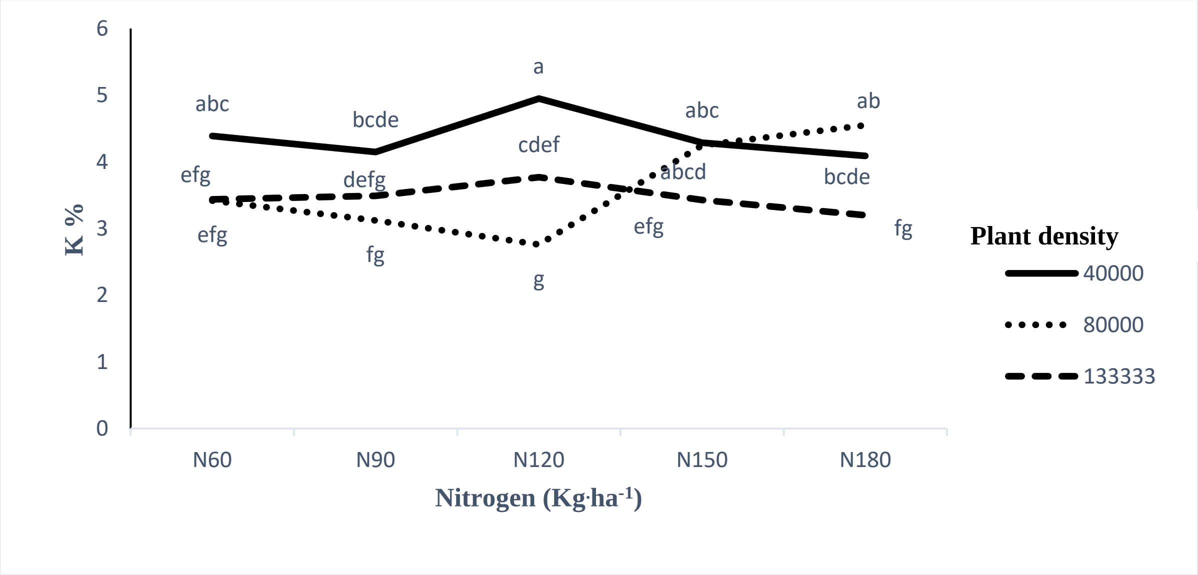

Figure 3.5 Interaction effect of nitrogen and plant density of leaf potassium (K) content of rocket. Points on lines with the same letter are not significantly different. Data in the interaction were analysed with Least Squares Means and means separated at $p \leq 0.05$ with Least Significant differences.

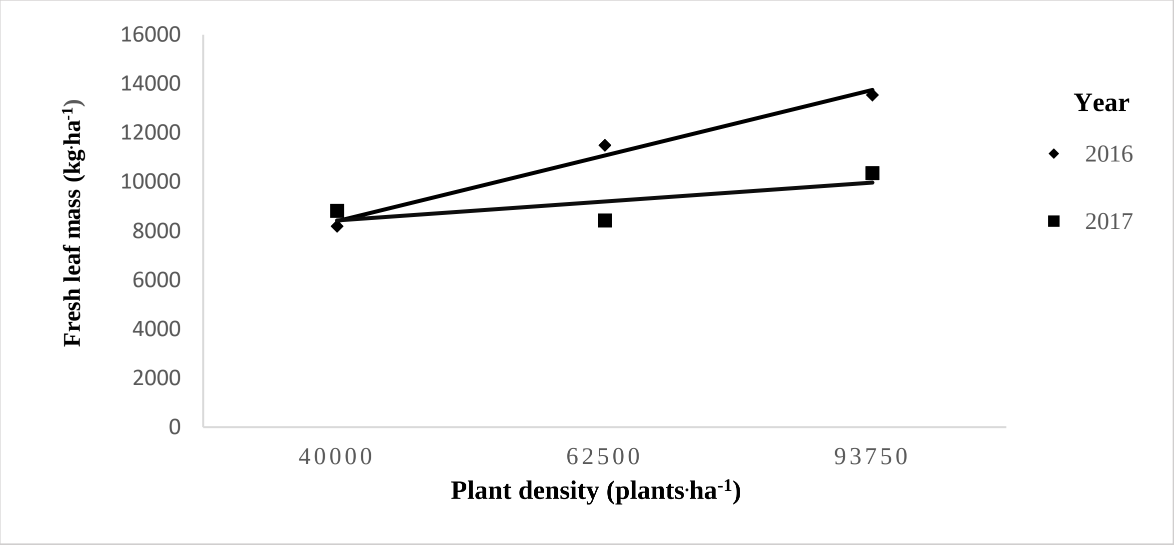

Figure 3.6 Effect of interaction of plant density and year on fresh leaf mass of basil.

Plant density of 93,750 plants·ha^{-1} resulted in greater fresh leaf mass of basil (Figure 3.6), although 2017 had lower fresh leaf mass than in 2016 at the highest plant density. A similar response occurred for the rocket, although differences between years began from the lowest plant density (Figure 3.7).

An increase in plant density resulted in increased leaf area and dry leaf mass of basil and rocket (Tables 3.4 and 3.5; Figures 3.8-3.11). The rocket's highest leaf area was at 133,333 plants·ha^{-1} for both seasons, although 2016 values for 80,000 and 133,333 plants·ha^{-1} were higher than in 2017 (Figure 3.8). There was an increase in leaf area and dry leaf mass for basil with increased plant density (Table 3.1, Figures 3.9 - 3.10). There was a similar increase in dry leaf mass of rocket with an increase in plant density in both seasons for 40,000 and 80,000 plants·ha^{-1}, but there was a decline in dry leaf mass at 133,333 plants·ha^{-1} in 2017 (Table 3.1, Figure 3.11).

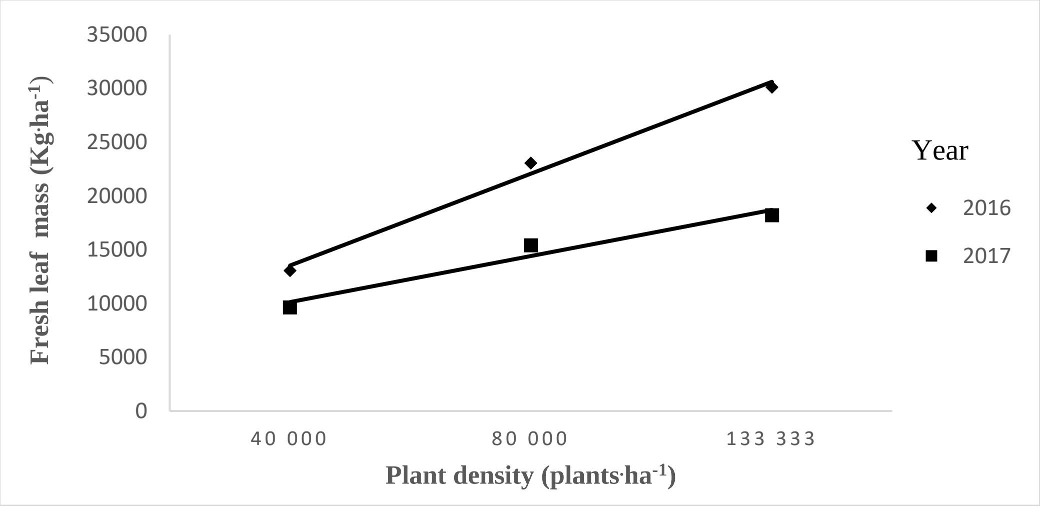

Figure 3.7 Effect of interaction of plant density and year on fresh leaf mass of rocket.

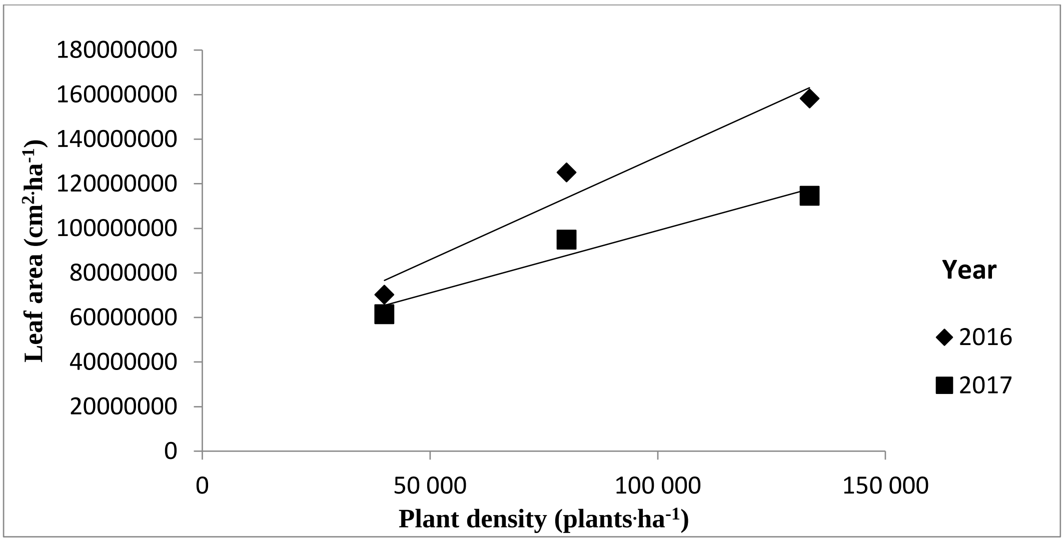

Figure 3.8 Effect of interaction of plant density and year on leaf area of rocket.

Leaf yield per unit area increased due to an increased number of plants. The highest fresh leaf mass of basil was due to increased plant numbers. Increased plant density for basil increased fresh shoot mass (Figure 3.12). Fresh shoot mass of rocket was higher in 2017 compared to 2016 at all plant densities and increased with an increase in plant density (Table 3.5, Figure 3.13). In 2016, differences in fresh shoot mass of rocket occurred between the lowest and highest plant densities (Figure 3.13).

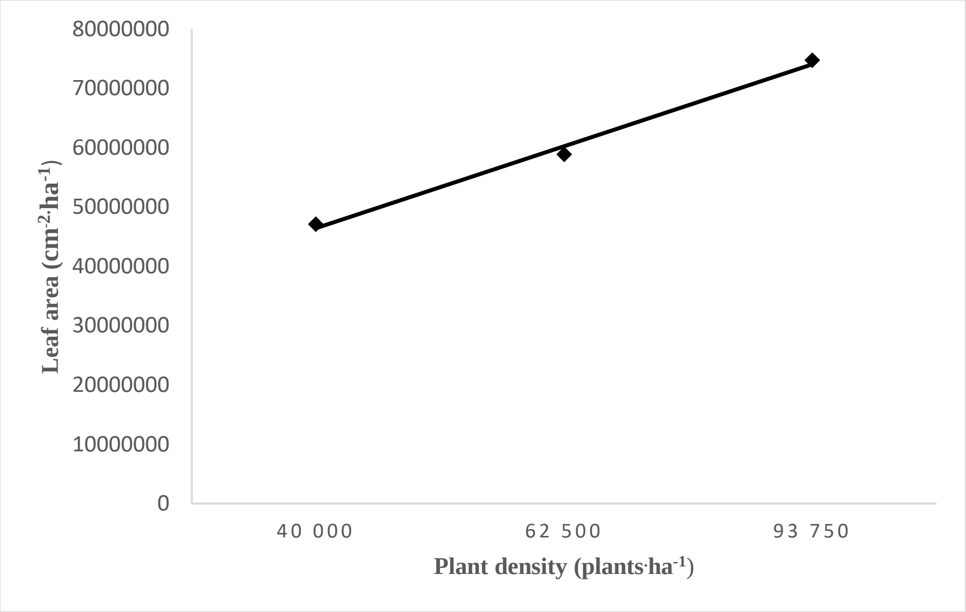

Figure 3.9 Effect of plant density on leaf area of basil.

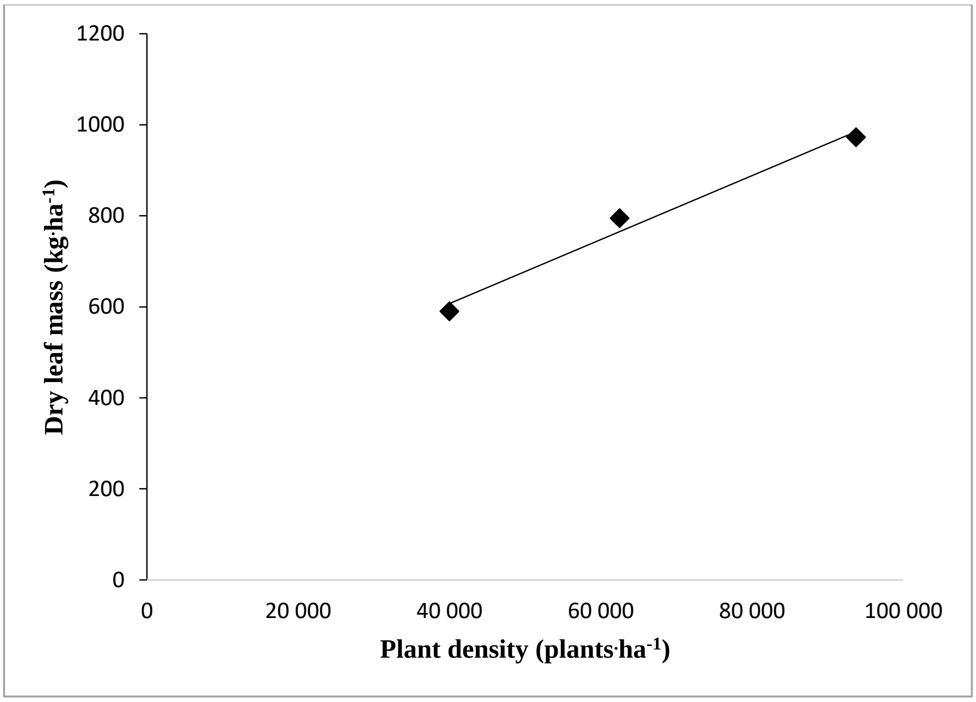

Figure 3.10 Effect of plant density on leaf dry mass of basil.

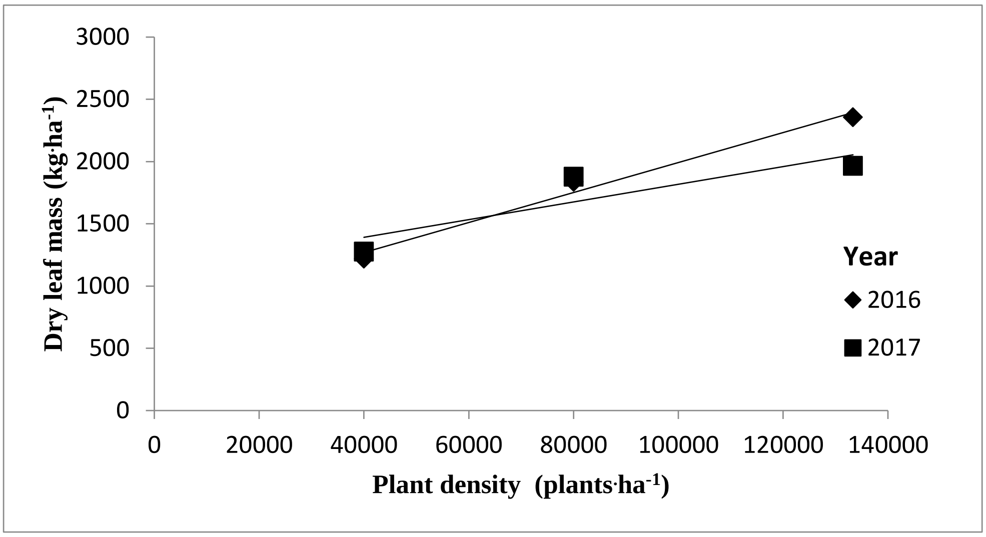

Figure 3.11 Effect of interaction of plant density and year on dry leaf mass of rocket.

Dry shoot mass of basil was higher at the highest plant density (Table 3.1, Figure 3.14). Dry shoot mass of rocket was higher in 2017 than in 2016 at all plant densities and increased with increased plant density (Figure 3.15). In 2016, differences in rocket dry shoot mass occurred between the lowest and highest plant density, with the highest plant density having greater dry shoot mass (Figure 3.15).

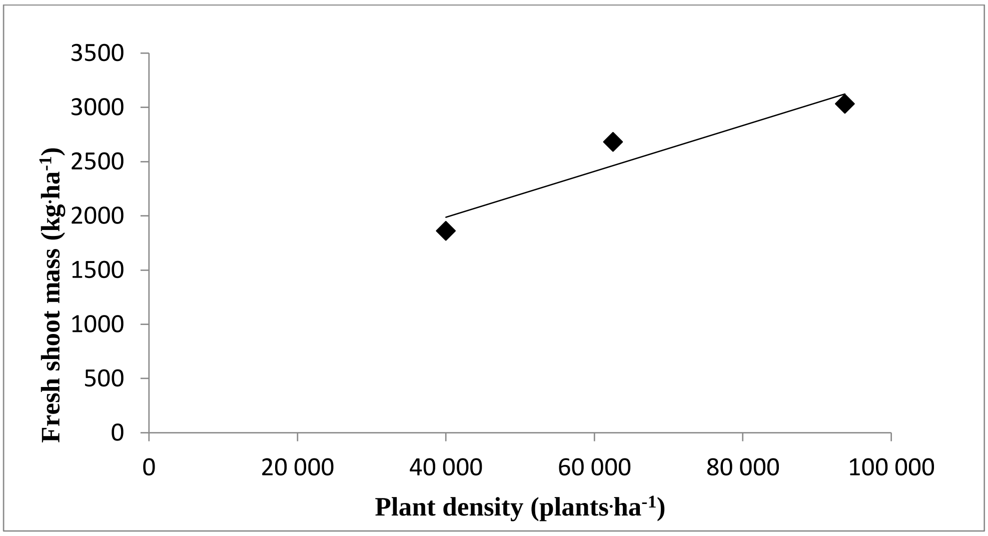

Figure 3.12 Effect of plant density on fresh shoot mass of basil.

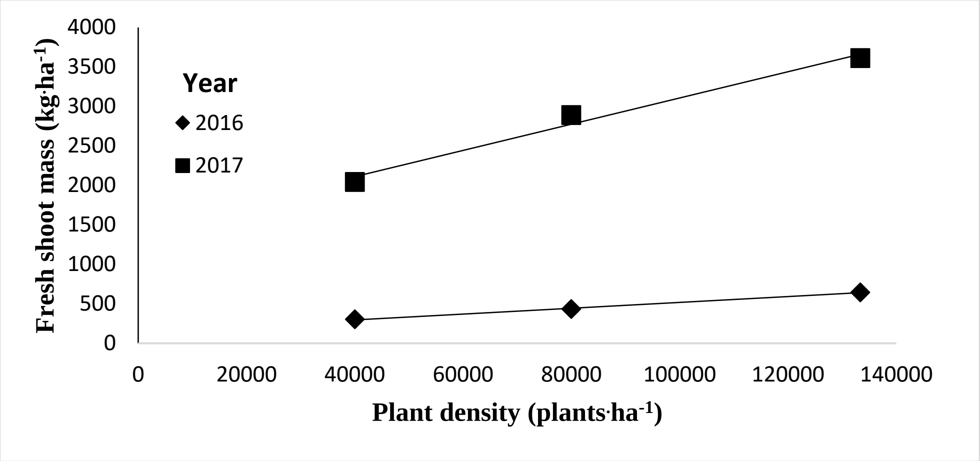

Figure 3.13 Effect of interaction of plant density and year on fresh shoot mass of rocket.

Table 3.6 Analysis of variance for the effect of nitrogen and plant density on basil leaf mineral content.

		N %	P %	K %	Ca %	Mg %
Source of variation	df[a]	MS	MS	MS	MS	MS
Rep	2	0.620	0.006285	0.2044ns	0.098907	0.003340
Nitrogen (N)	4	0.059	0.000231^{ns}	1.3156***	0.003237^{ns}	0.000456^{ns}
Plant density (D)	2	0.655***	0.004218^{ns}	0.1626^{ns}	0.091167***	0.026045***
N × D	8	0.123^{ns}	0.002573^{ns}	1.0653***	0.012417^{ns}	0.000586^{ns}
Error	28	0.083	0.002084	0.1668	0.008073	0.001489
Total	44					

*, **, ***Significant at 5%, 1% (p < 0.01) or 0.1%, ANOVA

Low fresh leaf mass of basil and rocket in 2017 (Figures 3.6 and 3.7) resulted in high fresh shoot mass of basil and rocket (Figures 3.12 - 3.13) and dry shoot mass of rocket (Figures 3.15). The relationship between high shoot mass resulting in low fresh leaf mass requires further investigation. There was no interaction between plant density and N on leaf N, P, Ca, and Mg contents of basil and rocket except for K leaf mineral content in both crops (Tables 3.6 and 3.7).

Table 3.7 Analysis of variance for the effect of nitrogen and plant density on rocket leaf mineral content.

		N %	P %	K %	Ca %	Mg %
Source of variation	df^{a}	MS	MS	MS	MS	MS
Rep	2	0.0616	0.025200	0.0239	0.25701	0.003396
Nitrogen (N)	4	0.8125**	0.012839^{ns}	3.5392^{ns}	0.16611^{ns}	0.000290^{ns}
Plant density (D)	2	0.1005^{ns}	0.009394^{ns}	0.2364^{ns}	0.01929^{ns}	0.000717^{ns}
N × D	8	0.1768^{ns}	0.011522^{ns}	0.9794**	0.05455^{ns}	0.002619^{ns}
Error	28	0.1126	0.064728^{ns}	0.2060	0.05535	0.003571
Total	44					

*, **, ***Significant at 5%, 1% ($p < 0.01$) or 0.1%, ANOVA

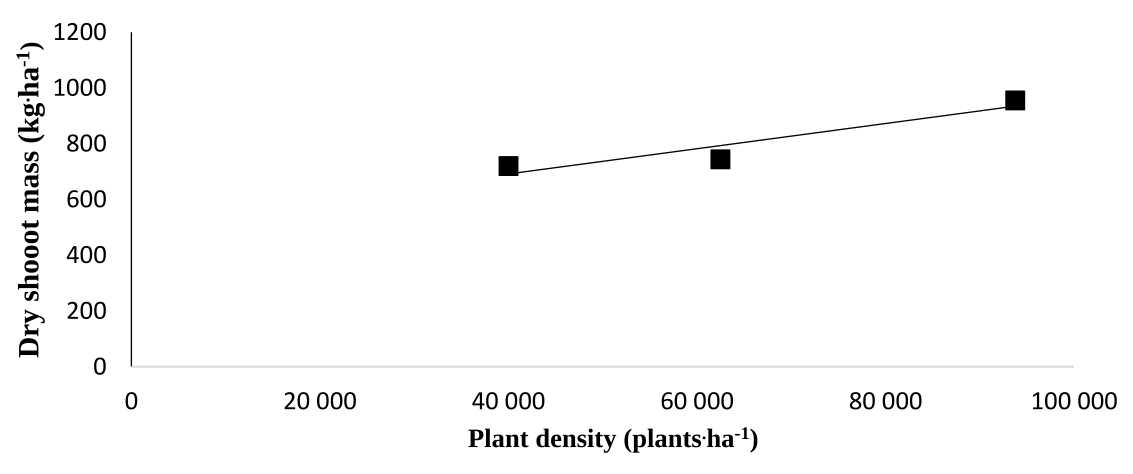

Figure 3.14 Effect of interaction plant density and year on dry shoot mass of basil.

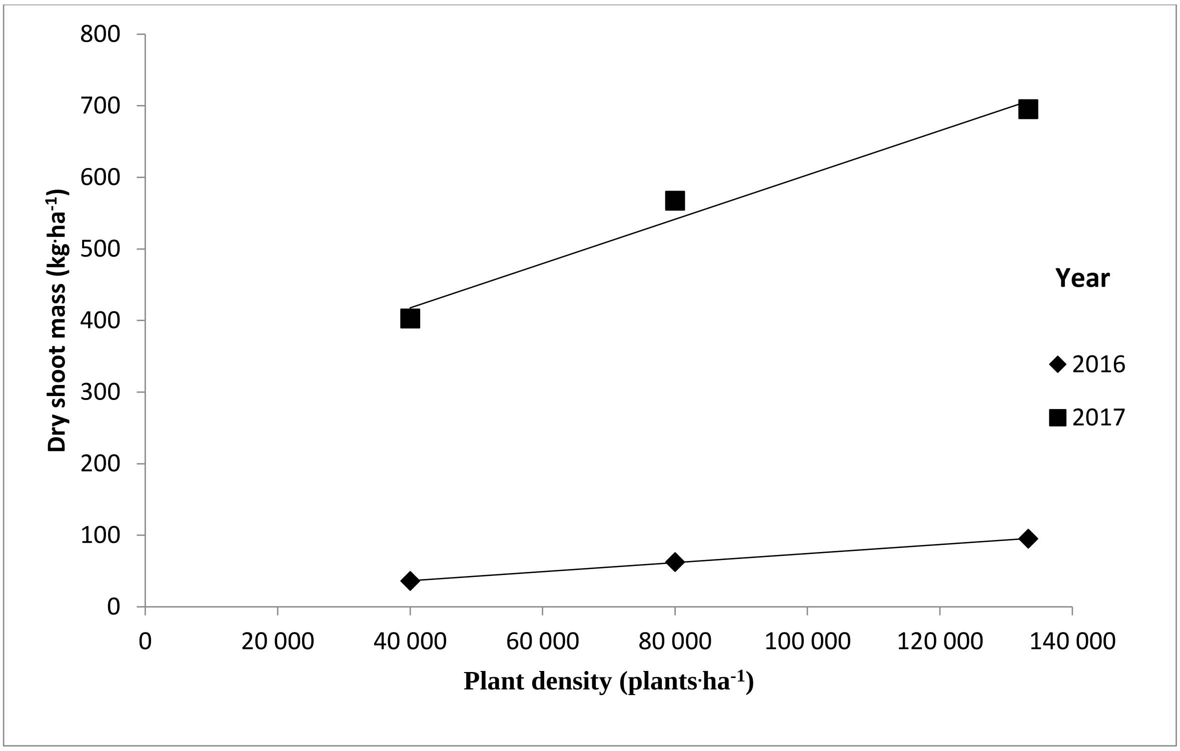

Figure 3.15 Effect of interaction plant density and year on dry shoot mass of rocket.

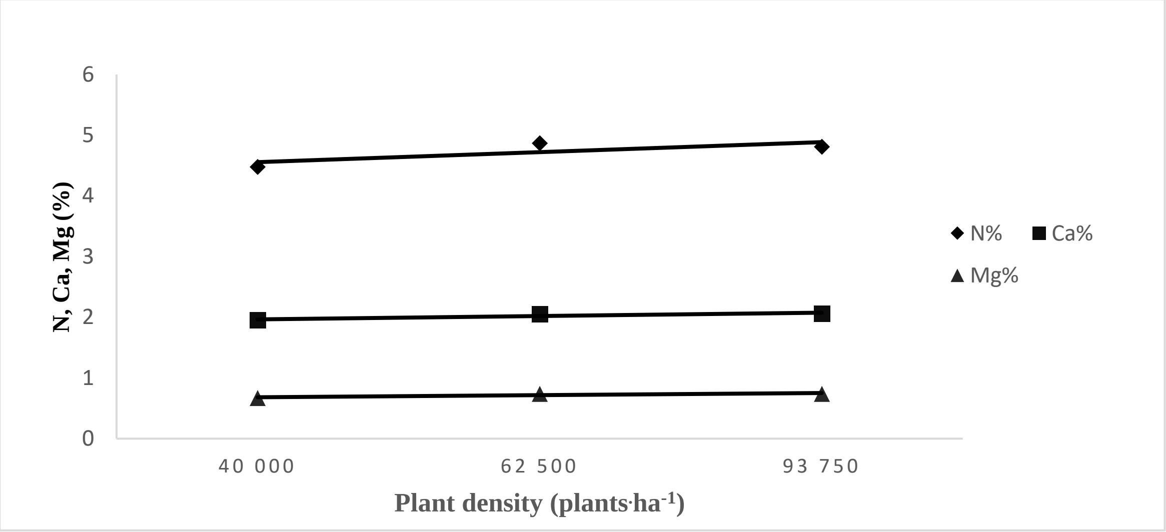

Figure 3.16 Effect of plant density on nitrogen (N), calcium (Ca) and magnesium (Mg) leaf content of basil.

Plant density affected leaf N, Ca, and Mg mineral content of basil and had a moderate positive correlation with plant density (Tables 3.1 and 3.6). Plant densities of 62,500 and 93,750 plants·ha^{-1} resulted in higher leaf N, Ca, and Mg content compared to a plant density of 40,000 plants·ha^{-1} on basil (Table 3.1, Figure 3.16). Plant density did not affect leaf P content for basil (Table 3.6).

3.5 Conclusion

Application of 60 kg·ha^{-1} N to basil at a plant density of 93,750 plants·ha^{-1}, and application of 60 kg·ha^{-1} N on the rocket at the plant density of 133,333 plants·ha^{-1} were suitable to improve yield. Further studies are necessary on N application rate and plant density above 93,750 plants·ha^{-1} and 133,333 plants·ha^{-1} to optimise plant spacing and N use efficiency of rocket and basil, respectively.

CHAPTER 4

Nitrogen levels, plant density and post-harvest storage duration affect phytochemical and antioxidant properties of field-grown basil and rocket crops

4.1 Abstract

Growers usually apply elevated nitrogen levels when plants are at high densities to obtain improved yields; how this practice affects phytochemical and antioxidant properties of basil (*Ocimum basilicum* L.) and rocket [*Diplotaxis tenuifolia* (L.) DC]) during postharvest storage, which are important quality aspects of these crops for human health, remains unknown. This study investigated effects of N application (60, 90, 120, 150, or 180 kg·ha^{-1}), plant density (40,000; 62,500 and 93,750 plants·ha^{-1} for basil; 40,000; 80,000 and 133,333 plants·ha^{-1} for rocket), and post-harvest storage duration (0, 5, 10 or 15 days) on phytochemical and antioxidant properties of basil and rocket. After harvest, the packaging of the leaves was in biaxially oriented polypropylene (anti-mist) bags and kept at 12°C and 85% relative humidity in a cold temperature room. Nitrogen application of 120 kg·ha^{-1} at day 0 of storage caused the highest accumulation of total phenolic content (TPC), flavonoid content (FC), strong-free radical scavenging activity, and % antioxidant with limited effect due to plant density on basil. In rocket, the application of 60 to 120 kg·ha^{-1} N at day 0 of storage had high TPC; FC was high at 90 to 180 kg·ha^{-1} N and 10 days of storage. Rocket had strong scavenging activity, due to 120 to 180 kg·ha^{-1} N, at 15 days of storage, and for

60 and 90 kg·ha^{-1} N at 0 and 10 days of storage. Storage time and N application affected the post-harvest quality of basil; TPC, FC, and free radical scavenging activity (FRS) reduced as storage time lengthened. Rocket had improved post-harvest quality at 10 days of storage. Spacing had limited effect on all parameters in basil and rocket. The most effective economic treatment in basil was 60 kg·ha^{-1} N for TPC, FC, FRS, and antioxidant activity in rocket was 120 kg·ha^{-1}, with a longer shelf life of 10 days of storage with regard to antioxidant activity. Optimisation of agronomic practises for improved production should consider phytochemical quality assurance to ensure there is no comprise in crop health benefits to consumers.

4.2 Introduction

Basil (*Ocimum basilicum* L.) and rocket [*Diplotaxis tenuifolia* (L.) DC.] are popular among consumers as green salads. Basil, an aromatic herb, is popular due its antioxidant, antimicrobial, and anti-inflammatory properties (Boateng, 2013; Marwat et al., 2011; Taie et al., 2010). Rocket contains vitamin C, carotenoids, glucosinolates and phenolics (Cavaiuolo & Ferrante, 2014; Hall et al., 2012b; Villatoro-Pulido et al., 2013). Their phenolic compounds are naturally occurring antioxidants, and their radical scavenging capability play an important function in reducing the risk of incidence of chronic diseases (Mata et al., 2007).

Bioactive compounds in plants can be affected by agronomic practices employed (Schreiner, 2005) and growing conditions (Nguyen & Niemeyer, 2008), without reducing marketable yield. Availability of plant nutrients is an important factor that influences secondary metabolism and antioxidant activities within plants (Stewart et al., 2001). Nitrogen application improves growth and yield of basil and rocket (Acharya et al., 2020; Mahlangu et al., 2020); it can decrease biosynthesis and accumulation of physiologically active substances, including flavonoids and phenolics, and have a further impact on produce quality (Groenbaek et al., 2016; Stewart et al., 2001). Nitrogen is involved in regulation of enzyme activity, photosynthesis, protein synthesis, antioxidant and osmolyte metabolism (Vanacker et al., 2006).

Plant density can be determined by spacing, and can affect availability of water, nutrients and exposure to light, which can influence crop quality (Marques, 2016). High plant densities reduce airflow around plant leaves, and under high humidity, can lead to fungal infection (Gilardi et al., 2012). Light availability to plants reduces at high-density, affecting plant growth, yield, and accumulation of phenolics (Bian et al., 2014). Environmental conditions before, and after harvest can alter phytochemical contents (Simmonds, 2003). The study investigated the influence of the levels of nitrogen fertiliser, plant density and post-harvest storage duration on total phenolic and flavonoid contents and antioxidant properties of basil and rocket.

4.3 Materials and Methods

The trial examined post-harvest aspects of work done with these crops where the yield was the primary focus (Mahlangu et al., 2020). The original trial was from January to May 2016 and repeated from January to May 2017. The original trial was arranged as a 5 × 3 × 4 factorial with N levels of 60, 90, 120, 150 and 180 $kg{\cdot}ha^{-1}$, and plant densities of 40,000; 62,500 and 93,750 plants·ha^{-1} for basil, and 40,000; 62,500 and 133,333 plants·ha^{-1} for rocket, and post-harvest storage duration of 0, 5, 10 or 15 days. The N application of 90 kg·ha^{-1} for both crops was the positive control according to soil analysis recommendations. Treatment combinations were replicated three times in a split-plot design. Harvested green leaves of basil (cv. Genovese) and the landrace rocket from each treatment were pre-cooled to 15°C before placing in biaxially oriented polypropylene bags (40 g per bag) with anti-mist coating and 9-µm perforations. The bags were stored at a non-chilling temperature of 12°C for basil (Cozzolino et al., 2016) and 10°C for rocket (Koukounaras et al., 2007), at 85% relative humidity. After each storage period, the samples underwent lyophilisation for further analysis.

4.3.1 Total phenolic content determination

Total phenolic content was determined using the Folin-Ciocalteu method (Makkar, 1999). The incubation of the reaction mix was for 40 min at 25°C. The recording of the absorbance readings was against a 50% methanol blank at 725 nm using a UV-Visible spectrophotometer (SPECORD® 210 Plus: Analytik, Jena, Germany). The experiment was in triplicate and total phenolic content calculated from gallic acid calibration curve.

4.3.2 Flavonoid content determination

Flavonoid content was determined using the aluminium chloride colorimetric assay (Moyo et al., 2013). The reaction mix was shaken, and absorbance readings recorded against a 50% methanol blank at 510 nm using a UV-Visible spectrophotometer

(SPECORD®). The determination took place in triplicate and flavonoid content calculated from a standard catechin calibration curve.

4.3.3 Free radical scavenging activity

Free radical scavenging activity was determined using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay (Sharma & Bhat, 2009). The sample was extracted using 50% methanol with sonication, placed inside a fume hood for a day and freeze-dried. The 50% methanol served as a negative control and ascorbic acid served as a positive control. The reaction mix was incubated in the dark at room temperature (25°C) for 30 min. Absorbance was measured with a UV-Visible spectrophotometer (SPECORD®) at 517 nm. The evaluation of each sample was in triplicate. Percent free radical scavenging activity was log-transformed and the EC_{50}, the effective concentration at which DPPH radicals are scavenged by 50%. The basis for the calculation of the free radical scavenging activity was the formula of Sharma and Bhat (2009).

$$\%\mathrm{RSA} = \frac{\mathrm{Abs(control)} - \mathrm{Abs\ (sample)}}{\mathrm{Abs\ (control)}} \times 100$$

4.3.4 Beta-carotene linoleic acid antioxidant assay

Antioxidant activity was determined using β-carotene linoleic acid antioxidant assay (Amarowicz et al., 2004). Each sample was prepared in triplicate and incubated in a water bath at 50°C for 60 min. After cooling the mix at 25°C, a UV-visible spectrophotometer (SPECORD®) recorded the absorbance at 470 nm. Butylated hydroxytoluene (BHT) was the positive control. The basis for the β-carotene bleaching calculation was the average β-carotene bleaching rate at 0 and 60 min. The overall antioxidant activity (%) by the extract was calculated based on the average β-carotene bleaching rate by the extract relative to the aqueous control (Amarowicz et al., 2004), using the following equation:

$$\text{ANT (\%)} = \left(\frac{Rcontrol - Rsample}{Rcontrol}\right) \text{ x } 100$$

4.3.5 Statistical analysis

Data underwent a 3-way analysis of variance (ver. 6.0, STSG Statistica for Windows, Statsoft Inc., Tulsa, OK) to determine effects of N application, plant density and post-harvest storage duration on basil and rocket physicochemical qualities. Significant interactions explained the results using Fisher's protected t-test least significant difference (Snedecor et al., 1980).

4.4 Results and discussion

The interaction between N, plant density and post-harvest storage duration were significant for phytochemical content (TPC and FC), free radical scavenging activity, and antioxidant activity in basil and rocket (Tables 4.1 to 4.2). The TPC decreased as storage duration increased in all treatments. On day 0 of storage (harvest), freshly harvested basil leaves receiving 120 kg$\cdot$ha^{-1} and 60 kg$\cdot$ha^{-1} of N, plant density of

62,500 plants·ha^{-1} and 93,750 plants·ha^{-1}, respectively, had the highest TPC (Figure 4.1).

Table 4.1 Analysis of variance for the effect of nitrogen, plant density and post-harvest storage time on basil phytochemical content and antioxidant activity.

Source of variation	df	Total phenolic content	Total flavonoids content	DPPH EC_{50}	% Antioxidant
		MS	MS	MS	MS
Residual	28	1.85	1.09	1671	7.84
Nitrogen (N)	4	536.65***	156.17***	39480***	180.172***
Plant density (D)	2	9.76 ***	6.58***	14213***	276.30***
N × D	8	742.74***	97.74***	24972***	182.07***
Time (T)	3	5668.88***	4712.46***	251040***	2142.03***
N × T	12	168.21***	62.18***	18829***	829.00***
D × T	6	10.65***	11.39***	14736***	84.48***
N × D × T	24	**103.69*** **	**88.11*** **	**30031*** **	**138.60*** **
Error	90	1.85	1.02	1597	8.71
Total	179				

*, **, *** = significant at 5%, 1% or 0.1%, ANOVA

df = Degrees of freedom, MS = Mean squares

The TPC in rocket was highest at 0 day of storage when plants received 60 to 120 kg·ha^{-1} N, with the highest value at plant density of 133,333 plants·ha^{-1} (Figure 4.2). At 93,750 and 133,333 plants·ha^{-1} of basil and rocket, respectively, there was more competition for availability of light, nutrients and water (Bjorkman et al., 2011), all of which possibly stimulate adaptive responses, such as increased phenolic content, resulting in high antioxidant activity. Degradation of TPC during post-harvest storage may be attributable to sensitivity toward oxidation (Rickman et al., 2007). An increase in high N negatively affects accumulation of phenolic compounds (Zhao et al., 2009).

Table 4.2 Analysis of variance for the effect of nitrogen and post-harvest storage on rocket.

Source of variation	df	Total phenolic content	Total flavonoids content	Free radical scavenging	Antioxidant activities
		MS	MS	MS	MS
Residual	28	0.049	0.09	11030	13.56
Nitrogen (N)	4	121.46***	14.86***	1311121***	700.82***
Plant density (D)	2	44.10***	37.14***	492264***	2.43
N × D	8	13.18***	11.22***	934903***	191.44***
Time (T)	3	571.77***	190.94***	5007170***	2991.35***
N × T	12	46.38***	30.34***	555932***	556.34***
D × T	6	14.96***	41.47***	571459***	31.65
N × D × T	24	18.47***	10.89***	970786***	285.08***
Error	90	1.05	0.58	98.48	14.56
Total	179				

The FC in basil was highest at 0 day of storage when treated with 60-150 kg·ha^{-1} of N, with limited effect due to plant density (Figure 4.3). Increase in storage duration reduced FC of basil. Conversely, rocket exhibited the highest FC at 10 days of storage and declined significantly at 15 days of storage across all N application rates (Figure 4.4) except for 60 kg·ha^{-1} N. Rocket fertilised with N at 60 kg·ha^{-1} had the highest FC at five days of storage. Application with 90 to 180 kg·ha^{-1} N produced high FC with increased storage duration to 10 days.

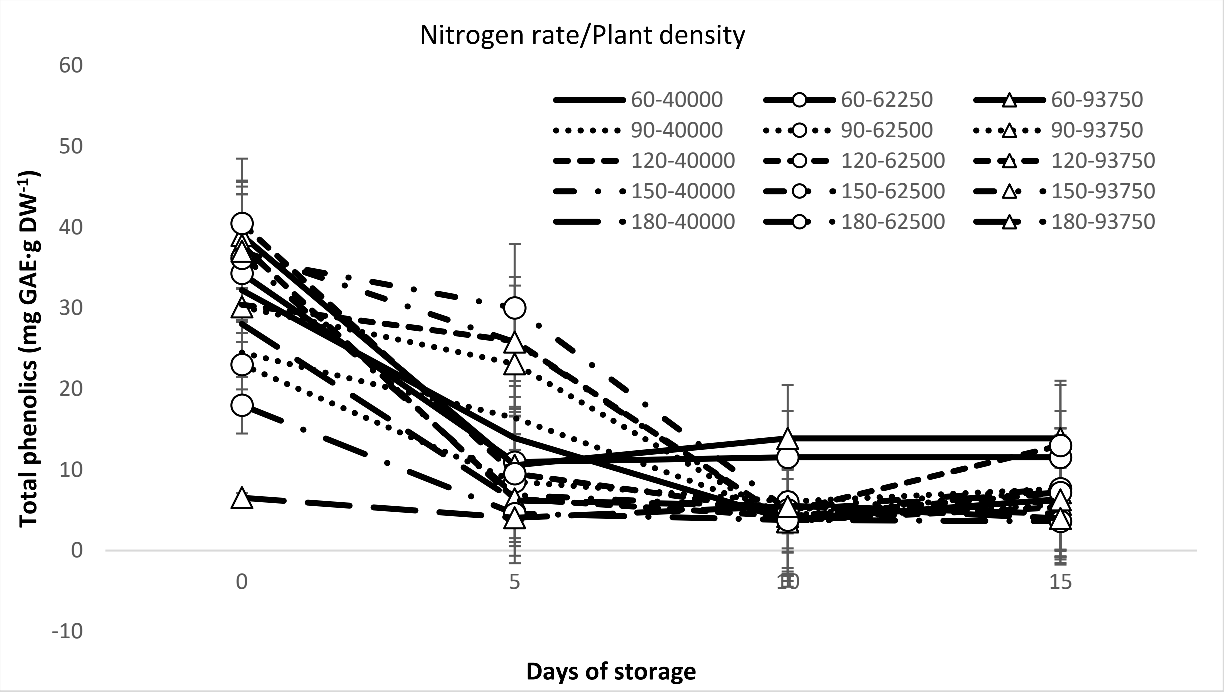

Figure 4.1 Interaction effect of nitrogen, plant density and post-harvest storage time on total phenolics of field-grown basil.

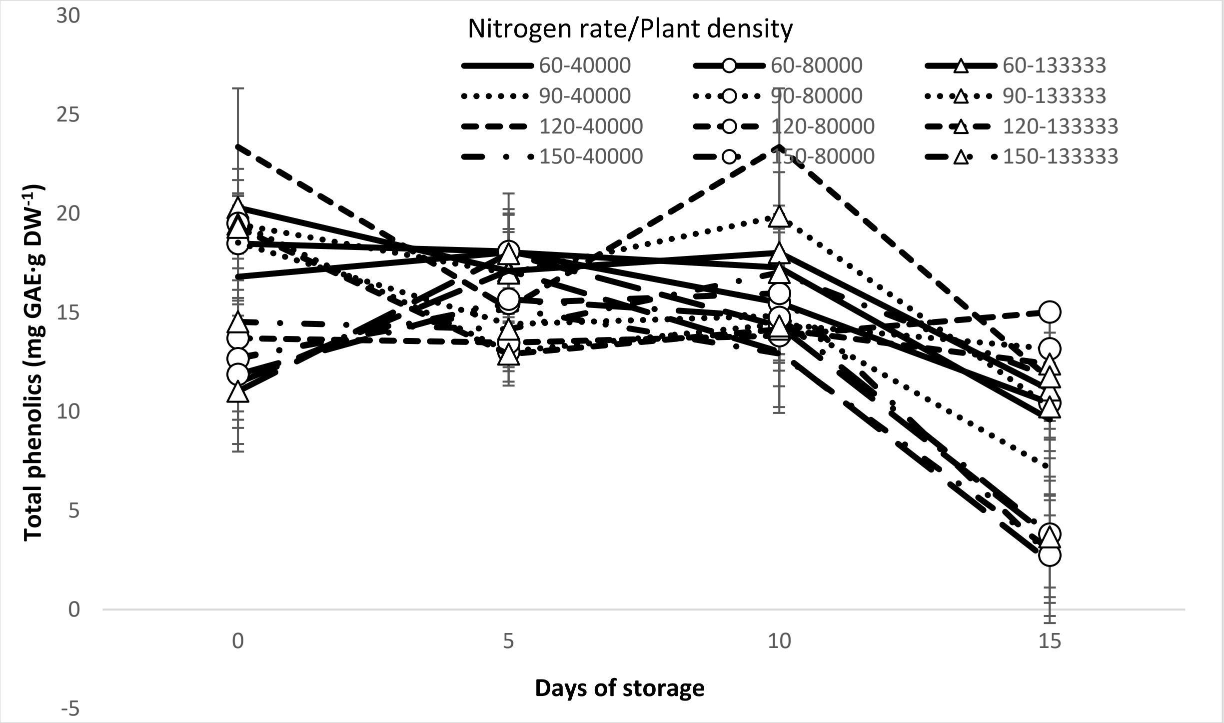

Figure 4.2 Interaction effect of nitrogen, plant density and post-harvest storage time on total phenolics of field-grown rocket.

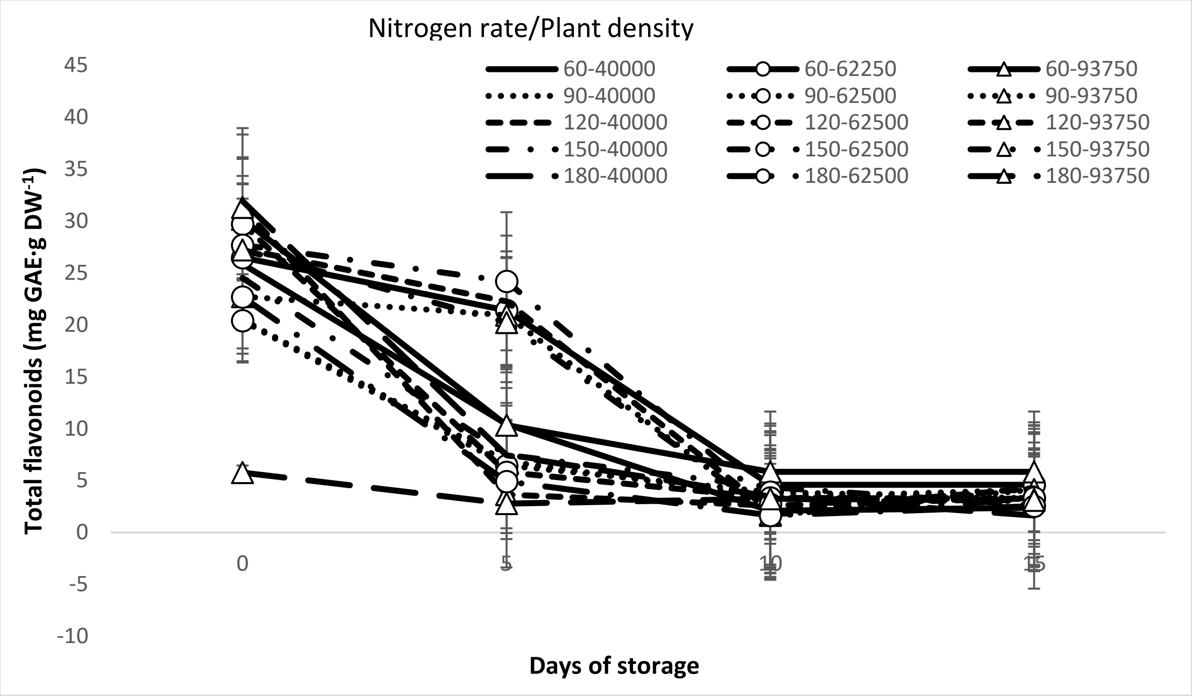

Figure 4.3 Interaction effect of nitrogen, plant density and post-harvest storage time on flavonoids of field-grown basil.

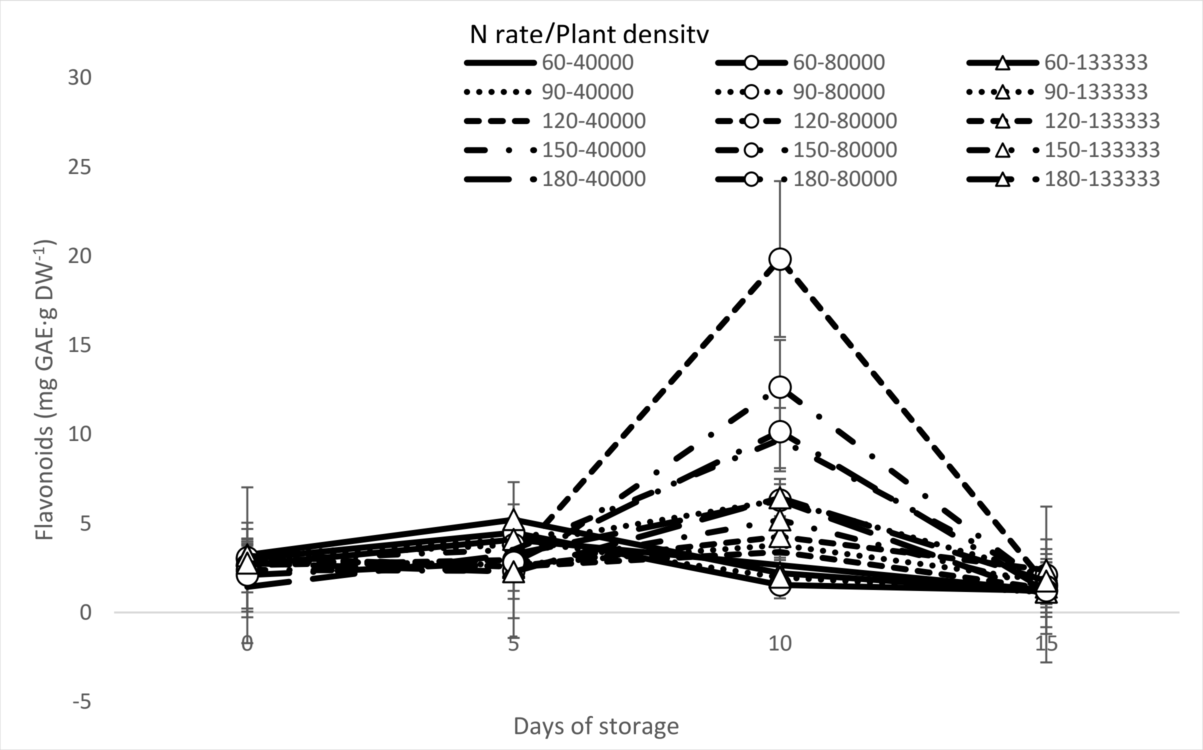

Figure 4.4 Interaction effect of nitrogen, plant density and post-harvest storage time on flavonoids of field-grown rocket.

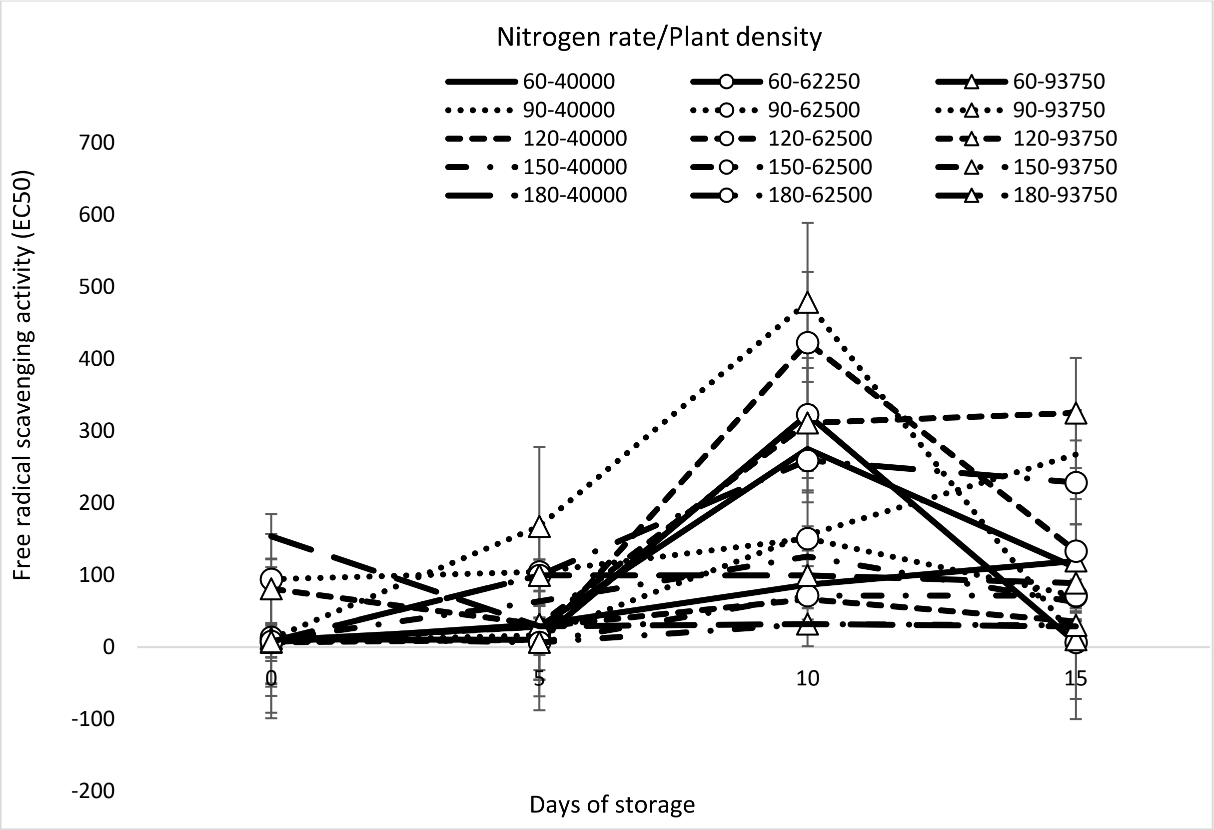

Figure 4.5 Interaction effect of nitrogen level, plant density and post-harvest storage time on free radical scavenging activity of field-grown basil.

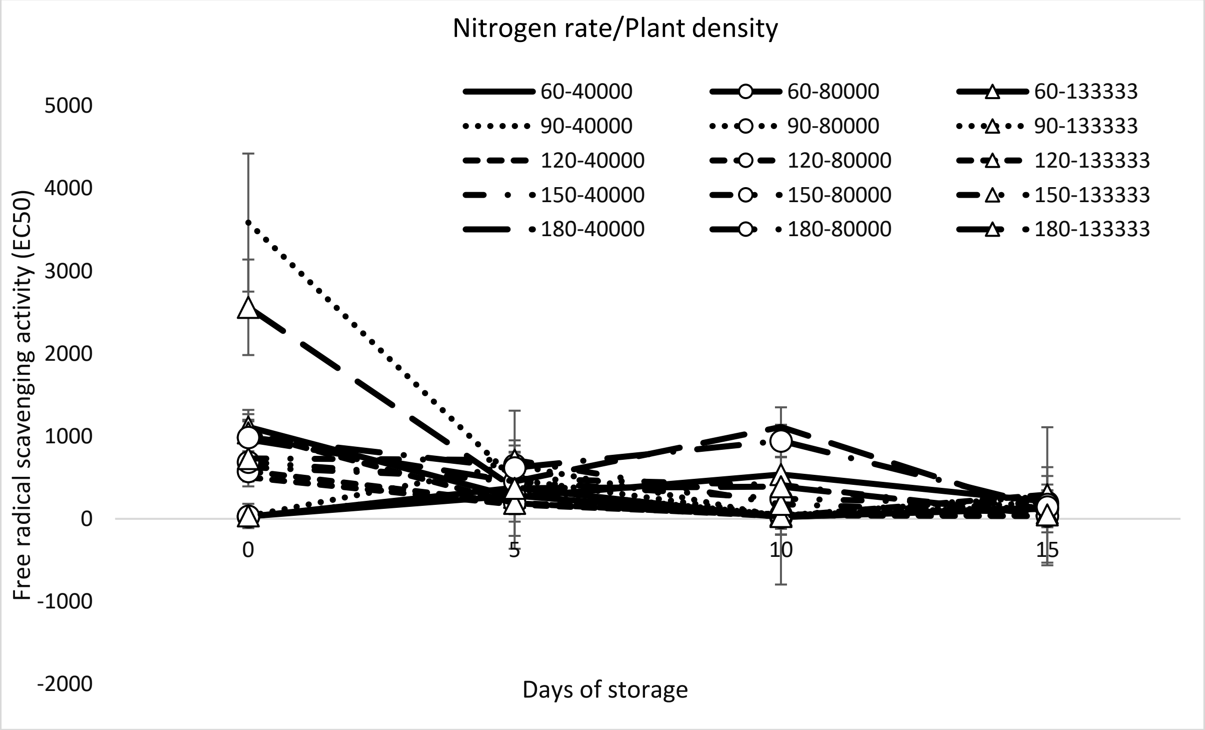

Figure 4.6 Interaction effect of nitrogen, plant density and post-harvest storage time on free radical scavenging activity of field-grown rocket.

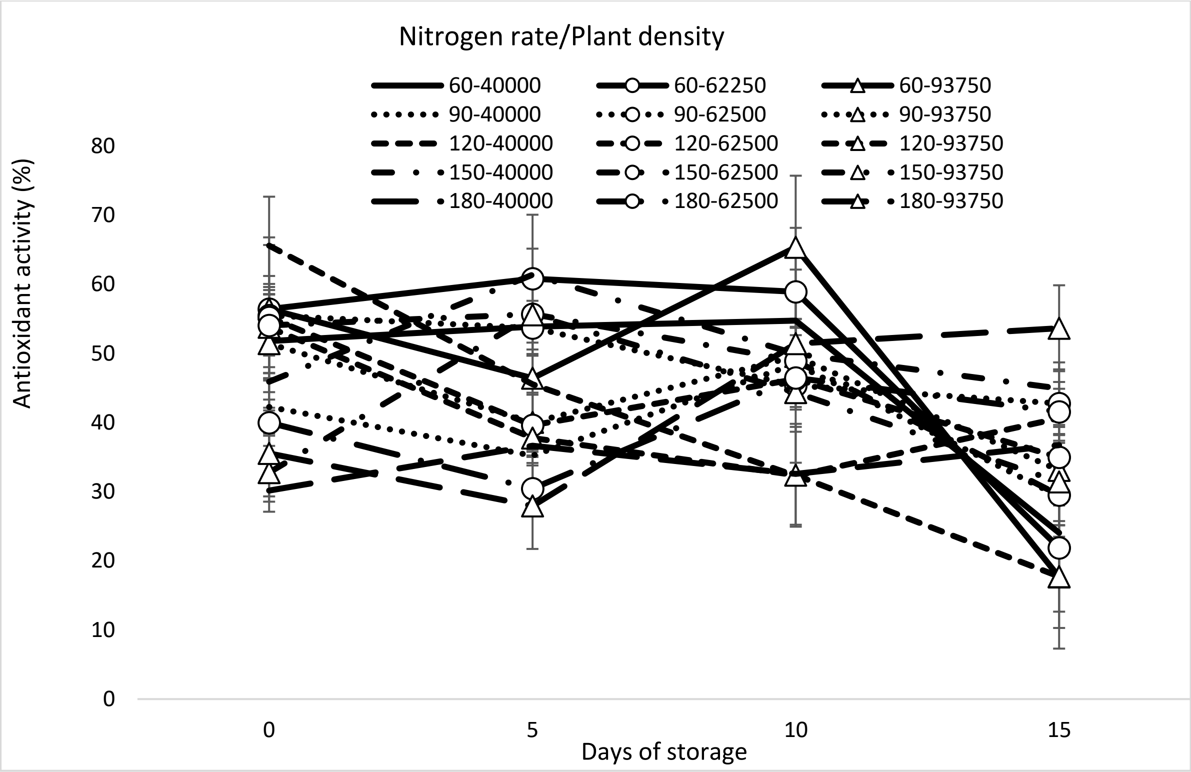

Figure 4.7 Interaction effect of nitrogen, plant density and post-harvest storage time on antioxidant activity of field-grown basil.

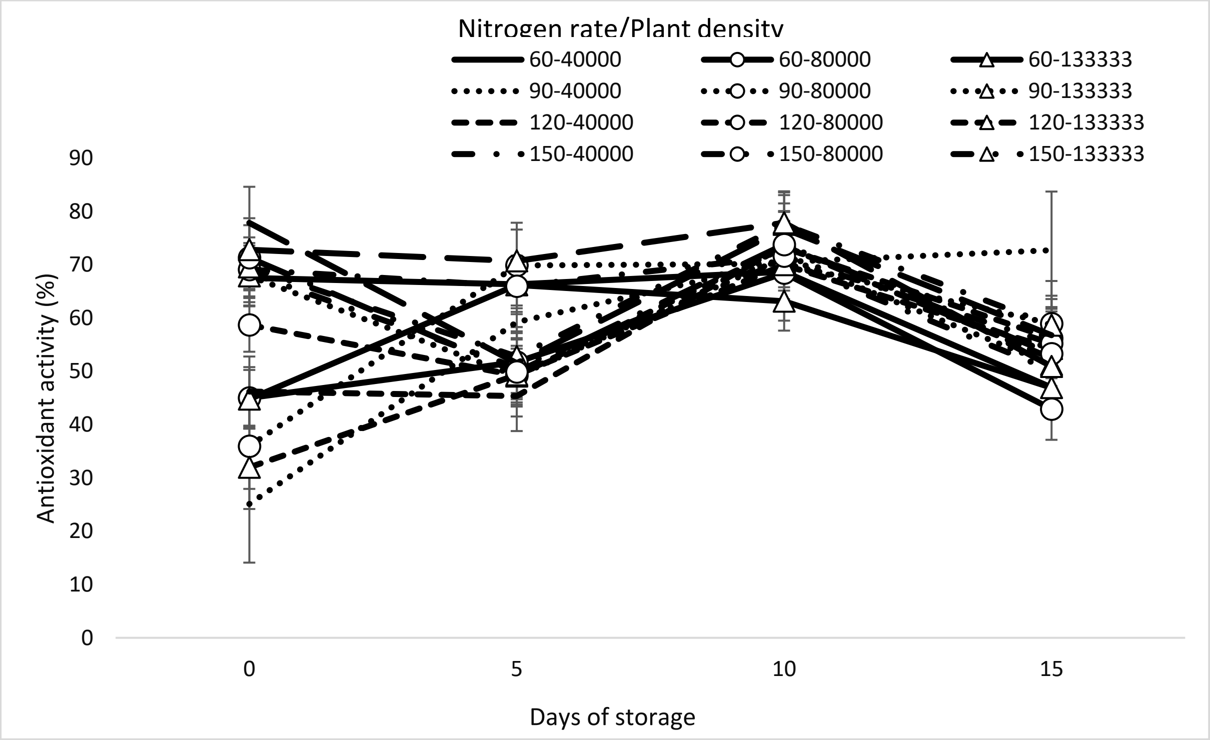

Figure 4.8 Interaction effect of nitrogen, plant density and post-harvest storage time on antioxidant activity of field-grown rocket.

Treatment affected free radical scavenging activity in basil and rocket leaves. The lower the EC_{50} value, the stronger the free radical scavenging activity (Amoo et al., 2012). The strongest free radical scavenging activity of basil was for all N and plant density treatments when stored from 0 to five days (Figure 4.5). Application 60 $kg \cdot ha^{-1}$ of N, at plant densities of 40,000 and 80,000 $plants \cdot ha^{-1}$ at 0, 10 and 15 days of storage had strong free radical scavenging activity in rocket (Figure 4.6).

Antioxidant activity of basil due to treatment with 120 $kg \cdot ha^{-1}$ N, at a plant density of 40,000 $plants \cdot ha^{-1}$ measured at 0 days of storage was high and similar to the 60 $kg \cdot ha^{-1}$ of N at 93,750 $plants \cdot ha^{-1}$ stored for 10 days (Figure 4.7). Antioxidant activity of basil was generally high at harvest (0 day of storage) due to treatment with 60 to 120 $kg \cdot ha^{-1}$ of N, which increased at five days of storage due to treatment with 150 $kg \cdot ha^{-1}$ N. Rocket had high antioxidant activity at 10 days of storage and N application of 60 to 180 $kg \cdot ha^{-1}$ (Figure 4.8). However, application of 180 $kg \cdot ha^{-1}$ N on rocket also had high antioxidant activity at 10 days of storage.

Previous findings recommended N be applied at 60 $kg \cdot ha^{-1}$ for basil and rocket at plant densities of 93,750 and 133,333 $plants \cdot ha^{-1}$ without compromising leaf yield (Mahlangu et al., 2020; Moyo et al., 2013). Rocket had strong scavenging activity at application 120 to 180 $kg \cdot ha^{-1}$ N at 15 days of storage, as well as 60 and 90 $kg \cdot ha^{-1}$ N at zero and 10 days of storage. Post-harvest quality of basil is sensitive to storage and N application above 120 $kg \cdot ha^{-1}$ while rocket had improved postharvest quality at 10 days of storage.

The most economical treatment in basil, based on fertiliser used, was 60 kg·ha^{-1} N on TPC, FC, FRS, and antioxidant activity in rocket was 120 kg· ha^{-1}, with a shelf life of 10 days of storage for antioxidant activity. Fertiliser application and spacing, and post-harvest storage duration can individually, and/or interactively, alter phytochemical properties and associated health benefits of basil and rocket. Optimisation of agronomic practises for improved production should consider phytochemical quality so that there is no compromise in crop health benefits to the consumers.

CHAPTER 5

5.1 Discussion and conclusion

Good agronomic practice (cultivar, N fertiliser, and plant density) plays the most important role in improving growth, yield and post-harvest storage duration. Agronomic practice focused on better management of fertiliser and plant population to maximise marketable yield, mineral content and phytochemical quality, preserve herbs for lucrative commercial markets.

Pre-harvest factors, such as N fertilisation rate, plant density and storage duration, can influence phytochemical content on basil and rocket. The pre-harvest practices may significantly affect storage duration of fresh-cut basil and rocket; lengthy storage duration is of high priority in fresh-cut herbs and leafy greens. It is important to determine whether good agronomic practices and proper storage conditions can represent a good source of phytochemical compounds in basil and rocket.

The results showed that application N regulates vegetative development and positively influence the yield, while low level of N application significantly reduced plant growth parameters, nutrient elements and phytochemicals content of basil and rocket leaves. Plant densities influenced the yield (leaf fresh, leaf dry mass leaf area, shoot fresh and shoot dry mass), and most of the leaf nutrient content was not affected. The high plant density showed a significant increase in the yield of basil and rocket, although post-harvest storage did not affect the phytochemicals.

Increasing N (120 kg·ha^{-1}) levels resulted in increased linearly leaf chlorophyll content of basil. Leaf chlorophyll content of rocket leaves was high, giving rise to fresh and leaf dry mass. The marketable yield production of basil and rocket did not differ significantly with the application of N, from 60-180 kg·ha^{-1}. An increase in N fertiliser application rate decreased leaf N content on rocket. Leaf K content was high with a low dose of N fertiliser (60-150 kg·ha^{-1}) and decreased at a high dosage of N fertiliser (180 kg·ha^{-1}); high dose of N fertiliser negatively affected the mineral content of herbs. At a low dose of N (N 60 kg·ha^{-1}), the phytochemicals content (TPC and FC) and antioxidant activities were high, except the free radical scavenging was high at a dosage of N 150 kg·ha^{-1} on basil. The TPC and free radical scavenging activities improved at a low level of N (N 60 kg·ha^{-1}) on rocket. The FC and antioxidant activities reduced with the low level of N and peaked at a high dose on rocket leaves. Medium plant density (62,500 plants·ha^{-1}) improved the phytochemicals content TPC, FC, and antioxidant activities of basil. A correlation between yield and phytochemical content (TPC and FC) and antioxidant activities were high in response to the N fertiliser rate. Increased N fertilisation resulted in greater depletion of phytochemicals and limited storage duration of herbs.

A good plant density for the plant is important in improving the marketable yield. Fresh leaf mass and leaf area of basil and rocket were low at low plant density (40,000 plants·ha^{-1}), and significantly increased at high plant density (93,750 plants·ha^{-1} basil and 133,333 plants·ha^{-1} rocket). Leaf area increases the rate of photosynthesis and produces a high dry leaf mass. Low plant density resulted in limited fresh and dry shoot mass and significantly improved at high plant density of basil and rocket respectively. Leaf K content improved at low plant density. The leaf mineral nutrients (N, Ca, and

Mg) rise with the accession of plant density. Leaf N, Ca, and Mg content was high at high plant density (93,750 plants·ha^{-1}) on basil leaves. N fertiliser rate and plant density on basil and rocket did not affect most of the leaf mineral nutrient content. Free radical scavenging was high at low plant density on basil. Rocket leaves showed high FC and free radical scavenging activity at medium plant density (80,000 plants·ha^{-1}), whereas TPC improved at 133,333 plants·ha^{-1} high plant density.

Correct storage and handling of vegetables maintain their freshness for longer and are safe for consumption. From freshly harvested basil leaves to storage of five days showed high free radical scavenging activity. Antioxidant activities of basil were generally high at harvest (0 days of storage) at a low level of N. The phytochemical content (TPC, FC, free radical scavenging and antioxidant activities) was high on fresh basil leaves and declined as the storage duration continued up to 15 days. In rocket leaves, the phytochemical content was low in fresh leaves and increased as the number of days increased. TPC, FC, and antioxidant activities were high in 10 days of storage and free radical scavenging was high at 15 days of storage.

Their interaction between N fertilisation rate, plant density and post-harvest storage duration was substantial in phytochemical content in basil and rocket. Freshly harvested basil and rocket leaves preserved TPC at high plant density; the lengthy storage duration decreases the TPC. The FC was high on freshly harvested basil leaves, and at a low level of N. Storing rocket leaves for 10 days in all levels of N improved the FC. The application of higher N rates reportedly decreases the storage duration. Storage of rocket leaves for 10 days increases the free radical scavenging activity and antioxidant activities. Plant density did not affect phytochemical content (TPC and FC), antioxidant activities and free radical scavenging.

The recommendation for improving the yield of basil and rocket is an application rate of 60 kg·ha^{-1} for nitrogen fertiliser; managing fertiliser application is economical for herbs production while increasing the yield. A high plant density of 93,750 plants·ha^{-1} for basil and 133,333 plants·ha^{-1} for rocket improved the marketable yield, and recommended for producing basil and rocket. Freshly harvested basil leaves showed high phytochemical content up to five days of post-harvest storage, while 10 days improved phytochemical content in rocket fresh leaves.

The study has shown that the cultivation practice of N application and plant density affects the yield, nutrients, chlorophyll content, phytochemicals and antioxidant content of basil and rocket. Pre-harvest N application rates had positive impacts on post-harvest storage of basil and rocket. The high plant density (93,750 plants·ha^{-1} basil and 133,333 plants·ha^{-1} rocket) increased the yield, increased plant density covered more soil with vegetation, and plants grew vigorous and healthier. The results indicated that the spacing did not affect the phytochemical, quality, and post-harvest storage time of basil. Basil and rocket applied with a low N (60 kg·ha^{-1}) rate resulted in maximum yield and preserved phytochemicals in lengthy post-harvest storage time. Lower N application rates influence antioxidant and phytochemicals with an increase in storage time of basil and rocket.

Further studies are necessary and need to focus on N application rate and plant density above 93,750 plants·ha^{-1} and 133,333 plants·ha^{-1} to optimise the plant spacing and N use efficiency of rocket and basil. Future research needs to focus on fertiliser application, amount of irrigation and fungal disease that is affecting the yield and reducing the quality during post-harvest storage duration.

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