Above ground effects of spacing on growth, performance and yield of container-grown spinach

by

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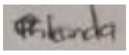
DECLARATION

I do hereby declare that dissertation “Above ground effects of spacing on growth, performance and yield of pot-grown spinach is work done by me under the guidance of Dr N.M Maluleke and Prof. L Khomo. The results of this dissertation have never been submitted to any Institution. All cited sources have been referenced as a sign of acknowledgement.



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Popular Chikanda

DEDICATION

I dedicate this dissertation to my husband Clapos Chikanda and my two sons Delight and Divine Chikanda. I thank you for tolerating me when I was busy with my studies at the expense of family.

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Firstly I give glory to God almighty who took me through. I say ‘Ebenezer’-this far the Lord has taken me.

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ABSTRACT

Container production of vegetable crops like spinach is becoming an easy and popular alternative of subsistence farming. However, there are several limitations of container plant production, which include space allocation. An experiment was carried out at UNISA to evaluate the effects of spatial configuration of pots on the growth, performance and yield of spinach. The experiment consisted of 15 quartets of spinach plants in pots with four distance treatments *viz.* 0 cm, 25 cm, 50 cm and 75 cm separating pots. Treatments were replicated as follows, 0 cm x 5, 25 cm x 5, 50 cm x 3 and 75 cm x 2. Parameters measured were leaf number, height, leaf area, chlorophyll concentration, stomatal conductance, dry leaf mass and dry root mass. Data was analysed using one way-ANOVA and the Tukey test was used to find means that were significantly different. Results demonstrated that spinach growth and development was significantly affected by the space allocated. The most leaves per plant (25 leaves), the tallest plants (348 mm), maximum leaf area (169 cm^2), peak leaf dry mass (39.5 g^{-1}) and maximum root growth (12.6 g^{-1}) were obtained at the 50 cm configuration. The zero cm spacing reported the least leaf area (79 cm^{-2}), least chlorophyll concentration (27 µmol m^{-2}), least stomatal conductance (1.9 m^2s^{-1}), least leaf production (21 g^{-1}) and most curtailed root growth (5.8 g^{-1}). There was a positive correlation between leaf number and plant height, any increase in leaf number caused an increase on the plant height. However, it was not so with other parameter, they did not have a significant association. Plant performance was limited at close range possibly in response to light competition. From the results of the study it can be surmised that plant growth, performance and yield increase as spacing

increase to a certain level and 50 cm spacing between planting pots appeared to be ideal for container-grown spinach studied here.

PFUPISO

Kurima mirivo yakaita sesipinachi mumavhasi kwave kukurumbira uye kunoita kuti vanhu vakwanise kurimira mhuri dzavo zvirinyore. Zvisinei, kune zvipinga mupinyi zvinosanganikwa nazvo pakuzama kurima mumavhasi zvinosanganisira kukura kwenhanho dzinopatsanusa mbeu kune dzimwe. Tsvakiridzo yakaitwa pachikoro chikuru cheSouth Africa (UNISA) cheRobert Sobukwe yekuongorora makuriro, negoho remurivo wesipinachi unorimwa mumavhasi. Murivo waive mumapoka gumi neshanu, boka rimwe chete riine zvirimwa zvina zvakasiyana nhanho pakati pembeu. Kubva mumapoka gumi neshanu, mashanu acho aive nembeu dzakabatana (0 cm x 5), mamwe mashanu aive nembeu dzakaparadzaniswa nenhanho makumi maviri neshanu (25 cm x 5), mapoka matatu aive nembeu dzakaparadzaniswa nenhanho makumi mashanu (50 cm x 3), mapoka maviri aive nembeu dzakaparadzaniswa nenhanho makumi manomwe neshanu (75 cm x 3). Zvakaongororwa muchidzidzo ichi zvaisanganira huwanda hwemashizha, kureba kwemurivo, kufaranuka kwemashizha, ruvara rwemashizha, kuvhurika nekuvharika kwenhengo dzemashizha, huremu hwemashizha akaomeswa uye huremu hwemidzi yakaomeswa. Zvakabuda mutsvakiridzo zvairatidza kuti chimiro chezvirimwa negoho racho zvirimaringe nekuparadzaniswa kwadzo mubindu. Murivo wakabatana (0 cm) wakange wakasarira kumashure pazvizhinji zvaisanganisa kukwikwidzana pamusaka pekuda zuva. Murivo waive nenhanho pakati pawo, kunyanya wenhanho makumi mashanu (50 cm) waikura zvakanaka uye goho rawo raive rakakura. Kubva muchidzidzo ichi kukura, mufaro uye goho rezvirimwa zvaiwedzerwa nekukura kwenhanho dzaiparadzanisa mavhasi adzo mubindu. Mushure meongororo iyi zvakaonekwa kuti kurima murivo mumavhasi akaparadzaniswa nenhanho makumi mashanu (50 cm) kunokurudzirwa.

Key words: spacing; containers; competition; allelopathy; growth; performance; yield

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Abbreviations

ANOVA	analysis of variance
CO_2	carbon dioxide
cm^2	square centimetres
df	degrees of freedom
GLV	green leaf volatiles
HSD	honest significant difference
mm	millimetres
MS	mean squares
NPK	nitrogen, phosphorus and potassium
r	Pearson correlation coefficient
S. oleracea	*Spinacia oleracea*
SS	sum of squares
VOC	volatile organic compounds
0 cm	no space
25 cm	single space
50 cm	double space
75 cm	triple space

CHAPTER 1: Introduction

Consumption of fruit and vegetables is the leading weapon against poor health, especially the leafy greens. They contain compounds with the ability to protect the body from many chronic and age-related diseases (Van Duyn and Pivonka. 2000). Their nutritional content gives the body a well-balanced defence and natural medicine for good living (Slavin and Lloyd, 2012).

Roberts and Moreau, (2016) stated that spinach is one of the leading leafy green vegetables, packed with many nutrients required by the body for growth and development. The crop has multiple uses and benefits though demand is high in some countries than supply (Cocetta et al. 2014), because of numerous factors such as space allocation to an individual plant, which has a direct impact on overall production. Therefore, understanding the effect of above ground space on pot-grown spinach, its physiological performances, growth and yield is of outmost importance to determine the optimum space required by plant to maximize yields.

Plant accessibility to space and resources influences growth, performance, and overall yield (Hussen et al. 2013). Plants to interact with other plants and the surroundings, despite being unable to walk and talk (Vanishri and Khandappogal, 2018). Associations which permit the transfer of both positive and negative information from one plant to another are formed either above ground or below ground, through the air, roots, and fungal networks (Cossin, 2014). Plant interactions which occur above ground are transmitted via volatile organic compounds (VOC) while below ground interactions are through roots, root exudates and hyphal networks (Vanishi and Khandappagol, 2018). Through these networks, plants flourish with conspecifics and aliens, as they attempt to fight against often harsh and potentially disastrous conditions (Falik et al. 2011).

Above and below ground plant vigour may be hampered by improper space allocation, which is influenced by over or under spacing (Trautz et al. 2017). The close spacing encourages competition for shortly supplied resources causing development of thin and tall plants (El-Badawy and Mehasen, 2012). However, wide spacing promotes growth and development, despite reducing number of plants per unit area, hence reduces yield. Therefore, there is need for optimal plant spacing, which can maximise growth, performance, and yield.

Several studies were conducted by Akinfasoye et al. (2008); Sikder et al. (2010); Maboko, (2012) and Islam et al. (2014) investigating the effects of space on field and hydroponic grown spinach crops. However, there is scanty information on space recommendations for container crop production. Production is done according to farmer preferences, even though spacing has a direct influence on plant growth and performance. The purpose of this study was to investigate how above-ground spacing affects growth, performance, and yield of container-grown spinach, so that productivity can be improved.

1.1 Problem statement

Spinach is one of the most important agricultural crops that has become a priority due to its high nutrient levels and bioactive compounds which are vital for the body. Container production is increasing, as people try to utilise every small piece of land available to maximise production and meet the requirements of the increasing demand. However, productivity is influenced by crop cultural management practises such as space allocation, which is a major practise that can affect both quality and quantity of production. Information on spatial recommendation of container-grown spinach has not been fully explored. Previous studies were conducted on space allocation for field-grown spinach, whereas container production spacing is done according to farmer choices and preferences, which is not based on scientific research. Plants are either under spaced or over spaced as producers try to maximize yields. Therefore it is important to study how variations in spatial arrangements of container-grown spinach affects crop growth, development, and yield production. Study findings will help to develop optimum spacing required for container- grown spinach to maximize yield production.

1.2 Significance of the study

Authors such as Bergquist, (2006), Miano, 2016 and Malchrzak et al. (2019) reported that spinach is rich in nutrients such as Vitamin A, E, B_6, C, K_1, and contain medicinal compounds such as carotenoids, flavonoids, polyphenols and phenolic acid which are known to play a pivotal role in human health. In South Africa, demand for spinach is higher than what the country can produce (Masufi, 2020). In the effort to increase production, backyard spinach container production is slowly increasing, despite insufficient information on proper container management practices. Spinach supply is affected by the number of plants grown per unit area (Law and Egharevba, 2009). Therefore, it was valuable to investigate the effects of above-ground spacing on the growth, and yield performance. From the findings of the study, 50 cm spacing is recommended as optimum because of its ability to perform better (in both growth and yield parameters) compared to other treatments used. An increase in production will fill the gap in nutritional needs of the ever-increasing population, subsequently improve livelihoods and reduce poverty through proper land space use.

1.3 Aim

The aim of this study was to investigate the effects of above ground spacing on growth, performance, and yield of container-grown spinach, so that suitable space allocation can be determined in order to improve productivity.

1.4 Objectives

- To determine plant growth parameters of spinach grown at varying spatial configurations relative to each other.
- To compare above and below ground yield of spinach grown at varying spatial configurations relative to each other.
- To determine ecophysiological performance of spinach grown at varying spatial configurations relative to each other.

CHAPTER 2: Literature review

2.1 Botany and origin of spinach (*Spinacia oleracea L*)

Spinach oleracea L. (*S. oleracea*) is an annual biparental herbage plant in Chenopodiaceae of the Amaranthaceae family (Koike et al. 2007). Reports suggested that it was first naturalized in Persia and was brought to North Africa by the Arabs, and then introduced to Europe. Currently it is produced globally, mostly in cool temperate regions (Department of Agriculture Forestry and Fishery, 2010). Spinach is believed to have arrived in China in the 7th century and the oldest written records of the plant were made in Iraq in the 14th century (Hallavant and Ruas, 2014). Evidence on how the plant spread globally and when it was first cultivated remain elusive. However, preserved specimen from the late 12th to early 13th century was found in the Pyrenees Mountain range at the border between France and Iberia (Ribera et al. 2020).

2.1.1 Cultivation of S. oleracea in Africa

Africa is not sufficiently exploited as the rich agricultural resource that it is. The north and southern part suffer repeated harsh weather conditions like droughts, floods, and land infertility (Cofie et al. 2010). The continent is further stricken by poverty; hence farmers are not able to constantly develop effective agricultural regimes, thus yields are always short of eliminating rampant food insecurity (Outéndé, 2020).

In South Africa, spinach production is relatively low compared to the potential, because it is considered still new in the commercial stake (Nemadodzi, 2015; Masufi, 2020). What is produced on a massive scale is Swiss chard, which is often mistaken for spinach, *S. oleracea* is the only true spinach. The country thinks of chard (*Beta vulguris* var. *Circla)* as spinach, but strictly speaking, it belongs to the beet family. This study focused on the above ground effects of spacing on container production of *S. oleracea* (spinach) sensu stricto.

2.1.2 Global production of spinach

China is the largest spinach producer worldwide, not surprising due to its population size, and it produces over 90% of the global stock. Its weather conditions are perfect for spinach cultivation (Ribera et al. 2020). Regarding seed production, Denmark leads, and is responsible for approximately 70% of the total supply of spinach seeds globally. Both China and Denmark have mild weather conditions and sandy loam soils, which are good for the production of spinach (Deleuran, 2011; Ribera et al. 2021).

2.2 Morphological characteristics of spinach

Spinacia oleracea has three different varieties, namely savoy, semi savoy and smooth leaf (Ma et al. 2016). Savoy type has big, crinkled leaves and low growth habit, while semi savoy type has an upright growth habit and a high disease and bolt resistance. The smooth leaf type has smooth leaves arranged in a rosette, with an ovate or triangular shape which is either narrow or long. Leaf sizes differ, they can be 30 cm long and one to 15 cm broad, with bigger leaves at the bottom and the smaller ones on the upper part of the flowering stem (Rubatzky and Yamaguchi, 1997).

Cultivar type and leaf thickness can influence yield production of the crops (Nayak et al. 2010). However, no relationship has been reported between leaf type and yield (Simko, 2014). The root system mainly consists of a thick taproot which has shallow fibrous roots close to the soil surface (Rubatzky and Yamaguchi, 1997).

2.3 Uses

Spinach is one of most important vegetables in the world, mostly produced for food and is rich in bioactive compounds such as carotenoids and flavonoids, making it a good additive for human health (Bergquist, 2006). Leaves are consumed fresh or frozen (Koh et al. 2012), as salads, cooked or consumed as soup and can be mixed with a variety of meals (Munts and Mulvihill, 2015).

2.3.1 Health benefits

Spinach is regarded as one of the essential foods which contains several positive health components which together prevents the effects of non-communicable diseases that kill people (Naseer, 2018). These benefits include (i) the prevention anaemia, (ii) contain anticancer agents, (iii) and ant-inflammatory agents. It can also alleviate the progression of several age related and cardiovascular diseases because of the presence of several nutrients, including vitamins such as (A, B, C, K) and trace elements such as iron and magnesium that are vital for the development of healthy bodies (Hedges and Lister, 2007). Rao et al. (2015) stated that the plant contains large quantities of folate, betaine, iron, calcium, potassium, folic acid, copper, protein, phosphorous, zinc, niacin, selenium and omeg-3 fatty acids, which are good for healthy living.

Miano, reported that *S. oleracea* has several therapeutic uses, with each 200 mg of spinach consumed containing 41 calories, while Malchrzak et al. (2019) (Table 2.1), revealed the composition per 100 g of spinach contains biochemical compounds such as flavonoids, carotenoids and phenolics, which act as antioxidants (Miano, 2016) (Figure 2.1). These antioxidants together scavenge free radicals that damage cell, protects the body from cardiovascular diseases, neurological disorders, cancers, obesity, eye problems, hypolipemic, regulates glucose levels in diabetics and they are good at improving skin texture (Roberts and Moreau, 2016). Compounds which are found in spinach helps in preventing age related muscular degeneration, haemophilia, anaemia and they reduce stroke risks (Al-gumboz et al. 2019). Spinach aqueous extracts also contain anti-inflammation effects that assist in wound healing, preventing ulcers and reversing wart development (Rubatzky and Yamaguch, 2012; Rahati et al. 2016).

Table 2.1 Compositional analysis of spinach (Malchrzak et al. 2019)

Spinach composition analysis	Content per 100 g
Energy value	23 kcal
Protein	2,9 g
Carbohydrates	3,6 g (including sugars 0,4 g)
Fat	0,4 g
Sodium	79 mg
Potassium	558 mg
Fibre	2,2 g
Vitamin A	781 µg
Vitamin E	1,4 µg
Vitamin K_1	400 µg
Vitamin B_6	0,2 g
Vitamin C	51 µg
Calcium	126 mg
Iron	4,1 mg

Figure 2.1 Graphical presentation of spinach and important nutrients (Miano, 2016).

2.4 Factors affecting growth and development

2.4.1 Planting and soil conditions

The Department of Agriculture, (2010) suggested that propagation of *S. oleracea* should be done through seeds, in wide beds arranged between 50-60 cm inter-row and 15-20 cm in-rows, though planting depth differs with soil texture, as heavier soils require shallow depths to promote germination. However, a good crop stand can be achieved through both the broadcasting method and the use of seedling trays. Spinach does well in different soil types, but well-drained saline sandy loam soils with low acidity are preferable, with pH ranging between 6-8 (Rubatzky and Yamaguchi, 2012). Hence soil sampling and testing is crucial prior to seed sowing.

2.4.2 Space effects on growth and development

Spinach requires various factors for growth, development and yield. For the crop to be able to fully utilize resources, proper cultural management is required (Weerakkordy, 2003). The leaf number, size, and growth habit are subject to the environmental parameters of the site and spacing (Rubatzky and Yamaguchi, 1997). Optimum spacing enables an increase in the number of leaves, branch development, great plant height and maximum biomass accumulation, both above and below ground (Sikder et al. 2010). Leaves of densely populated spinach plants usually fail to form a rosette and are likely to produce elongated shoots (Waseem and Nadeem, 2001).

Masombo (2018) reported that spatial arrangement has influence on the quality and quantity of produce. Lowly populated plants flourish well than highly populated, because of the absence of competition. An increase in plant density can also increase yields, though when too dense, resource competition is heightened, subsequently reducing plant growth and yield (Law and Egharevba, 2009). The optimum spacing is required to achieve better results, prompting the need for this study. There appears to be scanty knowledge on the above ground effects of *S. oleracea* grown in pots, another confounding factor demanding further study because most data on space were collected on field-grown plants. The present study investigated the aboveground effects of space on growth, performance and yield of pot-grown spinach.

2.4.3 Climatic conditions

Bergquiest, (2006) reported a case study on climate and leafy vegetable growth, yield quality and quantity. The findings showed that spinach requires much daylight via long days typical of summer in South Africa, but also thrives under cool temperatures. The savoy variety is considered the most tolerant of low temperatures. Rubatzky and Yamaguchi, (1997) indicated that seed germination is enhanced between 5-30 ^{0}C, though Koike et al. (2011) stated that 7-24 ^{0}C is the most suitable temperature, while rapid growth propulsion is possible between 15 and 18 ^{0}C. They further indicated that fully developed spinach plant can withstand low temperatures, but seedlings cannot tolerate freezing weather conditions. In addition, they remarked that long days combined with high temperatures promotes seed stalk development (bolting), which has a stimulating effect on yield quality and quantity.

2.4.4 Irrigation

Water management is one of the most important practices in *S. oleracea* production, since it is responsible for nutrient absorption, biomass and yield production yields (Zhang et al. 2014). Water stress tends to reduce plant pigment, quickening leaf senescence and photosynthesis (Vandoorne et al. 2012), while excessive watering may lead to plant death since there will be very little oxygen movement due to waterlogging (Halcomb, 2003). Spinach has a shallow rooting system, hence repeated application of light irrigation is required for better growth, development and yield (Bergquist, 2006).

2.4.5 Fertilizer

Optimal nutrient management in spinach production is highly recommended for pigment development in order to sustain the crop's high requirements (Heinrich, et al. 2013. This can be achieved by application of organic, synthetic fertilizers, or through combination of Fertilizers (Roy et al. 2009). Several researchers have assessed the impact of synthetic fertilizers, mainly nitrogen, phosphorous and potassium (NPK) and combination with bio-fertilizers (Islam et al. 2011, Zikalala, 2014; Elsady, 2016; Nemdodzi, et al. 2017 and Ogbaji et al. 2018). They demonstrated that plant growth and performance improved as fertilizer quantities increased. Fertilizers also influence crop yields, nutrient content and biochemical compound concentrations, although excessive application has a negative impact on the environment and structure of the soil, hence optimal application is required (Nemadodzi, 20115).

2.4.6 Harvesting

Simko et al. (2014) remarked that time and method for harvesting is determined by the purpose of production. Spinach is best harvested before the development of the seed stalk, either mechanically or manually by cutting the entire crown close to the root. For bagged/salad plants purpose, Koike et al. (2011) suggested that in California, harvesting should be done mechanically, earlier than regular spinach between 21-40 days after planting. However, these tender leaves obtain lower yields than the medium sized leaves, despite selling at high price. Furthermore, they reported that regular spinach produced for processing is left in the field to grow longer and thicker than the salad and harvesting can be done between 48-90 days after planting. Stage of harvesting should be considered always since it has a direct impact on yield quality such as biomass.

2.4.7 Postharvest handling and storage

Long ago, the outward appearance of fruits and vegetables was used to determine the period at which produce could be effectively stored before quality deteriorates (Ayala-Zabala, 2004). Spinach being a perishable vegetable crop that has a short storage life due to a high transpiration rate, it can easily be damaged by high temperature and long storage time (Mudau et al. 2015). Storability can be improved by rapid reduction of temperature. However, too low temperatures may lead to leaf discoloration and wilting (Nemadodzi, 2015).

2.5 Significance of container crop production

Container crop production started a thousand years ago, mostly for production of important plants like herbs, ornaments, and flowers. However, growers have several reasons for container plant production nowadays, namely (i) to save time, (ii) it requires less labour, (iii) it enables full utilisation of space available, (iv) it is easy to avoid poor soil conditions and better control of weeds (Walliser, 2017). It has become an essential ingredient in green houses, shade net and open space cultivation (shepherd, 2011) as well as in the nurseries using both soil and soilless cultures crop production. Crops can be grown throughout the year without worry about soils and weather conditions (Majsztrik et al. 2017).

Potting of crops is also associated with (i) easy management of moisture, (ii) light and temperature, (iii) less working of the soil, (iv) less pest and disease management and (v) easy transfer of plants to favourable conditions (Herda, 2010). However, farmers overlook the effects of spacing in container crop production, though it affects plant performance, yield quality and quantity. The present study fills the void on the above ground space effect on growth, performances and yield of *S. oleracea* grown in pots.

2.6 Plant response to spatial variation

Spatial arrangements and the number of crops per unit area is determined by the distance between plants and has an influence on plant behaviour and overall yield (Negash and Mulualem, 2014). Tempering with density and row spacing imposes competition for resources such as light, water and carbon dioxide. This competition subsequently inhibits light transmission through canopies and thereby, hampers photosynthesis, leading to debilitated morphology and physiology (Heitholt and Sasserath-Cole, 2010).

Plants can fight their battles in response to both abiotic and biotic stresses, despite being sessile (Brooker, 2006). They can eavesdrop their neighbours and react by the release of volatile organic compounds (VOC), through non-allelopathic or allelopathic volatile cues. These chemicals assist plants to send messages about the awareness of lack of space, resources and weather conspecific or other species are too close for comfort (Ramovs, 2013).

There are many plant VOCs, but the most important are (i) methyl salicylate, (ii) oxylipin metabolites, (iii) jasmonate, (iv) cis-jasmonate asterpenad and (v) green leaf volatile compounds (GLV). The GLV is the second largest group of plant signals after terpenoids, which occurs within and between plants (Matsui, 2006). Highly dense plant patches release VOC to avoid too many neighbours by trying to kill them and their response is identified by the changes in growth patterns (Pierik et al. 2004).

2.6.1 Competition types and definitions

Competition refers to the negative and positive interactions between two or more organisms of the same or different species striving to access resources (Willis, 2007). Many researchers such as Grime, (1973), define competition as “the tendency of neighbouring plants to utilize the same quantum of light, nutrient, molecule of water or volume of space”, in order to sustain the physiological needs. However, the most used definition for resource competition is “the process by which two or more individuals differently capture a potentially, common, and limiting resource supply” (Craine, 2009).

Gioria, (2014) defined negative interactions between plants for limited supply of resources as exploitative competition. The magnitude of the competition manifests differently in morphology. Craine, (2009) showed that if a resource is in short supply, both plants have difficulties in accessing it. The consequence of competition can be assessed between members of the same or different species Weigelt and Jolliffe, (2003), but this study focused on the same species.

2.6.2 Competition for light

Underground competition for nutrients and water, in container-grown crops is non-existent except for aboveground competition for resources, mainly light (Song et al. 2012; Seifert et al. 2014). Competition for light is uneven since light is directional, thus winners develop large stems, more leaf area. Plants with better access to light also grow leaves perpendicular to the source and develop interlocking canopies as the plants attempts to shade each other (Craine, 2009).

2.6.3 Intraspecific competition

Intraspecific competition is rivalry between individual members of the same species, negatively interacting with each other either direct or indirect for limited resources such as water, space and light (Lang and Benbow, 2013). This competition is influenced by the reduction in the supply of resources at an increasing demand. For sustainable yield quality and quantity, it is crucial to understand the mechanisms underlying competition (Craine and Dybzinski, 2013).

Several studies were conducted to determine the effects of spatial allocation of neighbouring plant and competition for available resources. For example, the studies by Heitholt and Sassenrath-Cole, (2009) and Wang et al. (2012) demonstrated that spatial arrangement impacts competition by altering plant growth, structure, form, shape, yield production and biomass accumulation.

An investigation by Makinde et al. (2009) on the effects of intraspecific competition on physiological characters of Jute, *Corchorus olitorius,* revealed a decrease in population growth rate as crop density increased. Findings of their study agreed with those of Makinde and Macarthy, (2006), who attested the significance of intraspecific competition and its effects on Cock's comp *Celesia argentinea*. They showed that an increase in plant population resulted in an increase in plant height, which confirmed the severity of intraspecific competition on plant growth.

Moniruzzaman, (2006) conducted a study to investigate the effects of plant spacing and mulching on yield and profitability of lettuce. Their findings demonstrated that yield parameters such as plant height and canopy width of field-grown lettuce increased with more spacing as compared to narrow spacing. Although this finding of more growth and increase in yield parameters is contradictory for plants under close spacing conditions because of high plant density per unit area.

Investigations by Sarkar et al. (2014) and Wang et al. (2005) asserts that plant physiological parameters increase at wide spacing but decrease at close spacing. Sarkar et al et al. (2014) obtained the highest leaf chlorophyll content at the widest spacing, but the least at closely spaced Water spinach *Ipomoea reptans Poir.* On the other hand, Wang et al. (2005) determined an exhibited reduction in stomatal conductance at densely spaced *Atriplex prostrata,* over widely spaced. All these studies determined that plant morphology, physiology, biomass accumulation and yield are influenced either positively or negative by intraspecific competition due to spatial configuration.

2.7 Allelopathy

Allelopathy existed for a long time without being defined. However, scholars believe that the subject began in 1937 after Han Molisch, a plant physiologist defined it

(Willis, 2007). He defined it as the interaction between plants through the emission of detrimental chemicals (Molisch, 1937). Rice, (1984) further defined allelopathy as uninterrupted or unintended positive or negative by-products of a plant or microorganism on another's territory, through chemical emissions. However, most authors defined it as forcible interactions exerted by one plant on another through emission of beneficial or detrimental substances which can affect plant growth and survival (Zeng, 2008).

2.7.1 Allelopathy and competition relationships

Allelopathy associations occurs directly or indirectly, but when the action involves competition, it is regarded as interference (Cheng and Cheng, 2015). Effects on plants are through gasses and exudes (Zeng, 2008). However, Del Moral, (1997) confirmed that allelopathy and resource competition are inseparable, but competition is the greatest form of interaction which is reflected by the emission of allelochemicals (An *et al*. 2013).

2.7.2 Possible routes of allelochemicals

Allelopathy depends on plant and environmental factors, of which variety and stage of growth are the plant factors, whereas environmental factors consist of biotic factors like (i) plants (ii) animals and abiotic factors like (i) soil, (ii) light and (iii) water (Rice, 1984). Allelochemicals are found in green, dead parts of plants like in the leaves, above and below ground stems, roots (exudes), seeds and flowers.

Volatilisation of chemicals can be transported through rain /irrigation water (de Albuqueque et al. 2010). Figure 2.2 below shows practical allelochemical pathways.

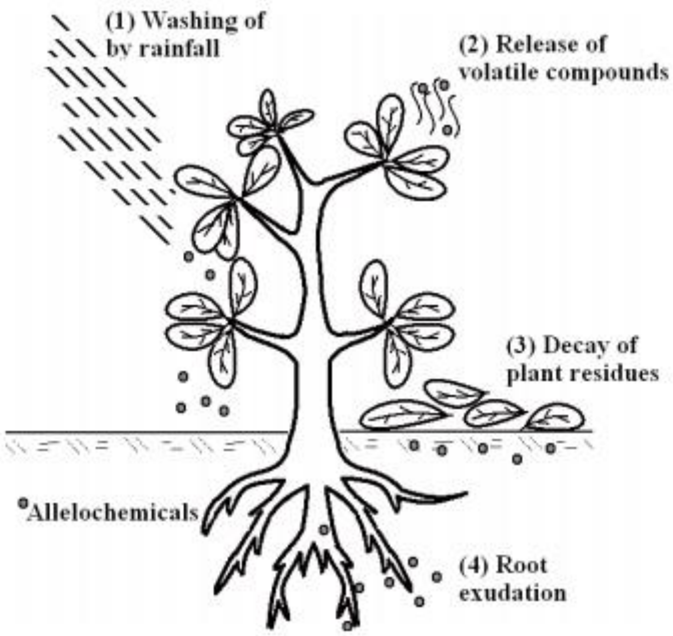

Figure 2.2 Potential allelochemical routes in plants (de Albuquerque et al. 2010)

2.7.3 Negative effects of allelopathy on plants

Volatile organic compounds hinder the function of plant organs by accelerating the; (i) decomposition of photosynthetic pigment, (ii) stopping energy and electron transfer. These result in reduction of enzyme activity, transpiration, stomatal conductance and inability to photosynthesize (Wu et al. 2004). Zeng, (2014) stated that allelochemicals also inhibits easy access of soil nutrients, subsequently cause harm to soil microbes (Huang et al. 2013). Condor and Indrea, (2010) investigated the possible allelopathic in spinach and red beet. They found out that planting red beet after spinach leads to severe losses. Spinach leaves harmful allelochemical in the soil, which reduces and slows seed germination, increases mortality and reduces the mass.

2.7.4 Allelobiosis

Allelobiosis is the chemical interaction between undamaged plants of the same species, exchanging communicative messages to each other, though the receiver's response might affect the growth and trophic strategies, by increasing plant ability to acquire resources like light (Ninkovic et al. 2006). Plant adaptation to allelobiosis can be through more biomass allocation, either above or belowground (on shoots or roots) (Ninkovic, 2010). Li et al. (2016), stated that the causes are non-toxic and may remain neutral to the receiving plant unlike allelopathy cause, which are toxic and may cause harm to the receiver. Allelobiosis and allelopathy are inseparable, and both can affect plant performance at once. However, allelobiosis has not yet received enough attention. Figure 2.3 below shows allelobiosis interaction in undamaged plant and its effects physiological and morphological parameters.

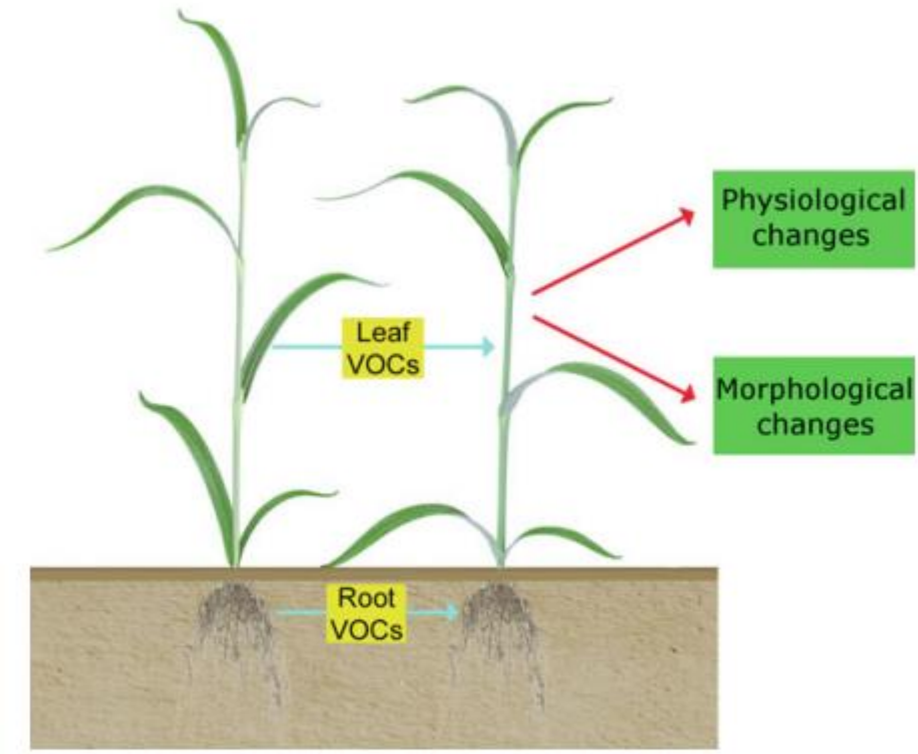

Figure 2.3 Plant-plant communication of undamaged plants through VOC (allelobiosis), affecting physiological and morphological parameters (Ninkovic, 2010).

2.7.5 Positive effects of allelochemicals

Allelochemicals can positively affect the environment by replacing synthetic pesticides, insecticides and herbicides which are hazardous to the environment and continuously developing resistance (Ihsan et al*.* 2015). For example, root exudates of *sorghum bicolour* can successfully prevent weed growth, without affecting other crops (Uddin et al. 2014). Therefore, sustainable innovative measures which seeks to optimize spatial arrangements with ability to improve plant growth, vigour, and yield, at the same time being environmentally friendly, especially in pot grown crops, are required.

CHAPTER 3: Materials and Methods

3.1 Experimental site

The trial was conducted at the University of South Africa (UNISA) Robert Sobukwe Campus (Figure 3.1), in Johannesburg, Gauteng (GPS coordinates 26.1586^0 S, 27.9033^0 E). The area is 1753 m above mean sea level and it receives an average of 700 mm of rain per annum. The weather is mild during winters (May to August) and warmer from October to March.

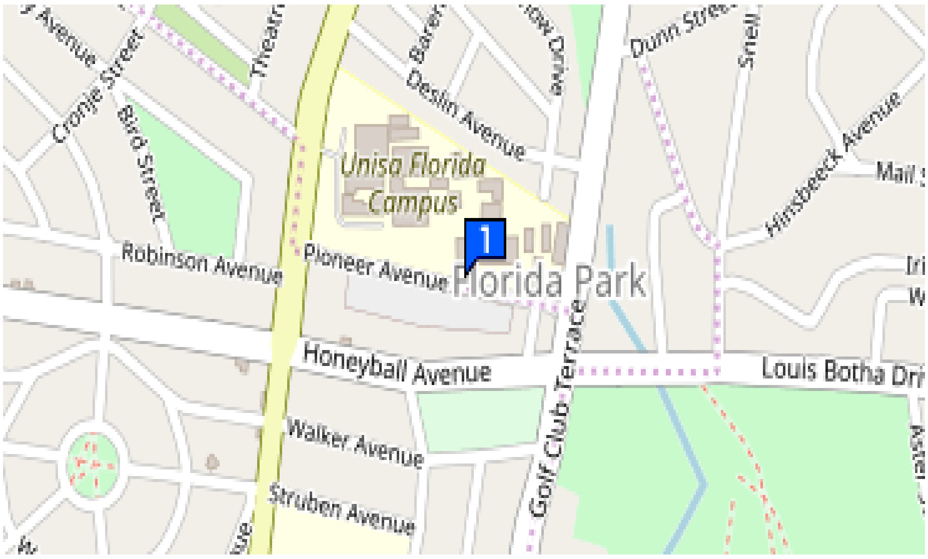

Figure 3.1 Experimental site in Florida, Roodeport, Johannesburg where the pot trial took place.

3.2 Experimental design and layout

There were 60 plants grown, comprising 15 sub-plots of four plants each, planted randomly in four different spatial arrangements (Figure 3.2). The first arrangement was no separation of the four plants, this was replicated five times, the second was a 25 cm separation which was also replicated five times, the third arrangement was 50 cm distance between the four plants replicated thrice, and finally a 75 cm spacing between the plants replicated twice (Table 3.1 and Figure 3.2). Plant arrangement consisted of 3 rows and 5 columns, which were grouped and labelled from A to O (Figure 3.2).

Table 3.1 Experiment treatments and replication

Treatment (cm)	**Number of replications**
0	5
25	5
50	3
75	2

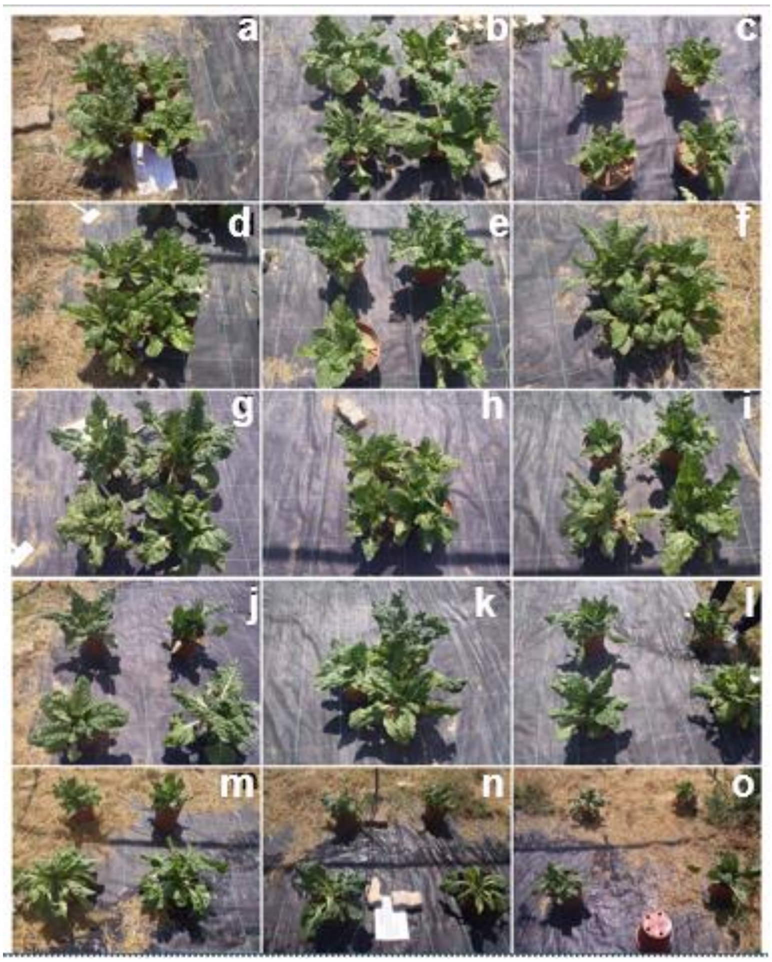

Figure 3.2 Improvised layout of plants in the field showing separation of individual plants in each group from group A to group O. For example, group E are four pot plants separated by 50 cm while plants in O are 75 cm apart.

Savoy certified spinach seeds by Starke Ayres (Pty. Ltd), were planted on seed trays filled with peat (TS1) and then placed under greenhouse conditions for 30 days. The greenhouse temperature (using digital thermometer) was kept between 15 and 25 ˚C, the optimal range for spinach seeds in general, and the relative humidity (Anden Wall Mount Digital Humidistat) was maintained between 60 and 75% using automated aerial sprinklers. Germination occurred a week after sowing and only well-established and vigorous seedlings were transplanted, four weeks after seeding. Seedlings were transplanted to 30 cm × 30 cm pots, which were placed in the field and allowed to grow for nine weeks. The plants were monitored and managed continuously throughout the growing season. Nutrients were applied in the form of granule fertilizers (10 grams/pot) every 5th day, during the experimental period. Throughout the growing season, plants were watered manually using a watering can, according to the soil moisture requirements detected by a 3-way meter which measures the moisture content of the soil, light intensity and pH levels. Scouting of pests and diseases was done regularly to protect the crops from damage.

3.3 Data collection

Plant growth and development was monitored throughout the experimental period with regular measurements of height and leaf number. Plant height was measured using a meter ruler and after nine weeks the final height was recorded. The number of leaves on each plant were physically counted. The adaxial chlorophyll concentration and abaxial stomatal conductance were measured in five representative leaves per plant. The stomatal conductance was measured with an AP4 Leaf Porometer which measures the diffusion conductance across a leaf by comparing the rate of humidification in its chamber to readings obtained with the calibration plate. The plate has six diffusion calibrated settings whose values are known precisely from finite element analysis. After all measurements were taken, plant yield was determined by measuring the dry mass. All the leaves and roots from each plant were harvested and dried for later weighing. They were placed in paper bags and put in an oven for 72 hours at 40˚C. A sub-sample of the leaves was mounted onto graph paper and scanned to determine leaf area (Figure 3.3). Leaves were traced out in the graphics package Inkscape where the number of squares was computed by image analysis.

Figure 3.3 Spinach leaves countered algorithmically and mounted on graph papers to determine the area.

All data collected was subjected to analysis of variance (ANOVA). The statistical analyses were done using R software, which was developed in the early 1990s, by Ross Ihaka and Robert Gentleman in New Zealand. One-way ANOVA was used to determine whether there were statistically significant differences between means of the various parameters and treatments. The Tukey HSD (Honest Significant Difference) was also performed to test for differences among the sample means for significance. Strengths and relationships between variables were measured using the Pearson correlation test.

CHAPTER 4: Results

4.1 Introduction

The results are presented according to the study objectives and the findings revealed that different spatial configurations of container-grown spinach produced variations in growth, performance and yield (Tables 4.1, 4.2 and 4.3). The response of plants was different from one treatment to another, which means to grow, develop, or produce *S. oleracea* plants requires optimum spacing.

Table 4.1 One-way ANOVA results for physiological and biomass parameter

Source of variation	df	SS	MS	F	P-value
Chlorophyll	3	703	234.3	3.083	0.0277
Error	292	22193	76		
Total	295	22896			<<0.05
Conductance	3	119.2	39.72	18.55	4.87E-11
Error	292	625,4	2.14		
Total	295	7446			<<0.05
Leaf number	3	185.9	61.97	1.803	0.157
Error	292	1890	34.36		
Total	295	2075.9			>>0.05
Plant height	3	45494	15164.6	3.788	0.0153
Error	55	220173	4003.1		
Total	58	265666.8			<<0.05
Leaf area	3	316882	105627	11.27	5.94E-05
Error	244	2286110	9369		
Total	247	2601784			<<0.05
Leaf mass	3	2407	802.3	1.917	0.138
Error	52	21762	418.5		
Total	55	24169.13			>>0.05
Root mass	3	357.5	119.2	3.644	0.0184
Error	52	1700.5	32.7		
Total	55	2057.98			<<0.05

df= degrees of freedom, SS= sum of squares, MS=mean squares, f= f-ratio, p-value=0.05

4.1 Response of Chlorophyll to variations in spatial arrangement

The study findings (Table 4.2) present the effect of different spatial configurations on the leaf number, leaf area, chlorophyll content, stomatal conductance and plant height of pot grown *S. oleracea*. Findings show that spatial arrangement of pots had a significant influence on the chlorophyll concentration in the leaves as shown graphically in Figure 4.3. Chlorophyll concentration ranged from 26.9 $\pm$ 9.7 to 30.6 $\pm$ 8.6 µmol.m^{-2}. The highest chlorophyll concentration of 30 $\pm$ 8.6 µmol.m^{-2} was attained at 25 cm spacing, while the least was 26.9 $\pm$ 9.7 µmol.m^{-2} at 0 cm spacing. The 50 cm spacing and 75 cm spacing obtained 29 $\pm$ 8 and 28.7 $\pm$ 7.6 µmol.m^{-2}, respectively.

Table 4.2 Spinach growth and physiological parameter responses to different spatial arrangement of pots

Spacing (cm)	Mean leaf number/plant	Mean leaf area (cm²)	Mean chlorophyll (µmol.m^{-2})	Mean conductance (mmol m^{-2} s^{-1})	Mean height (mm)
0	21.3 $\pm$ 6.1	79.5 $\pm$ 57	26.9 $\pm$ 9.7	1.9 $\pm$ 0.8	282.8 $\pm$ 76.6
25	21.5 $\pm$ 6.6	135.6 $\pm$ 102	30.6 $\pm$ 8.6	2.2 $\pm$ 1.7	310.5 $\pm$ 66.7
50	25.4 $\pm$ 5.2	169 $\pm$139	29 $\pm$ 8	3.3 $\pm$ 1.7	348.2 $\pm$ 40.9
75	20 $\pm$ 3.6	87.8 $\pm$ 86	28.7 $\pm$ 7.6	3.4 $\pm$ 1.6	264 $\pm$ 40.8
	NS	S	S	S	S

Means with S letter are significant at $p = 0.05$ and means with NS are non-significant at $p = 0.05$

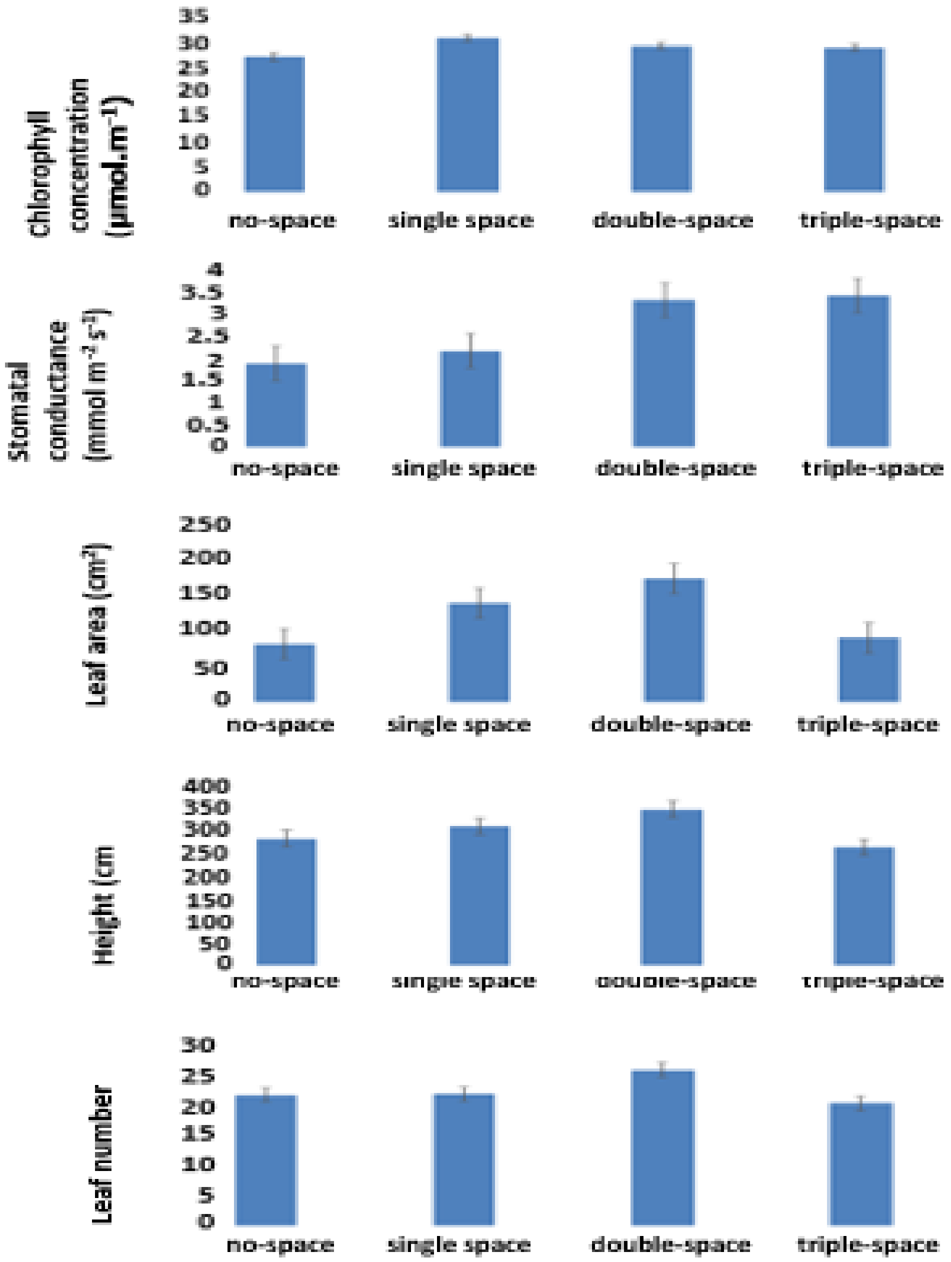

Figure 4.1 Plant growth and physiological parameter and space relationship. Single space =25 cm, double space =50 cm and triple space = 75cm.

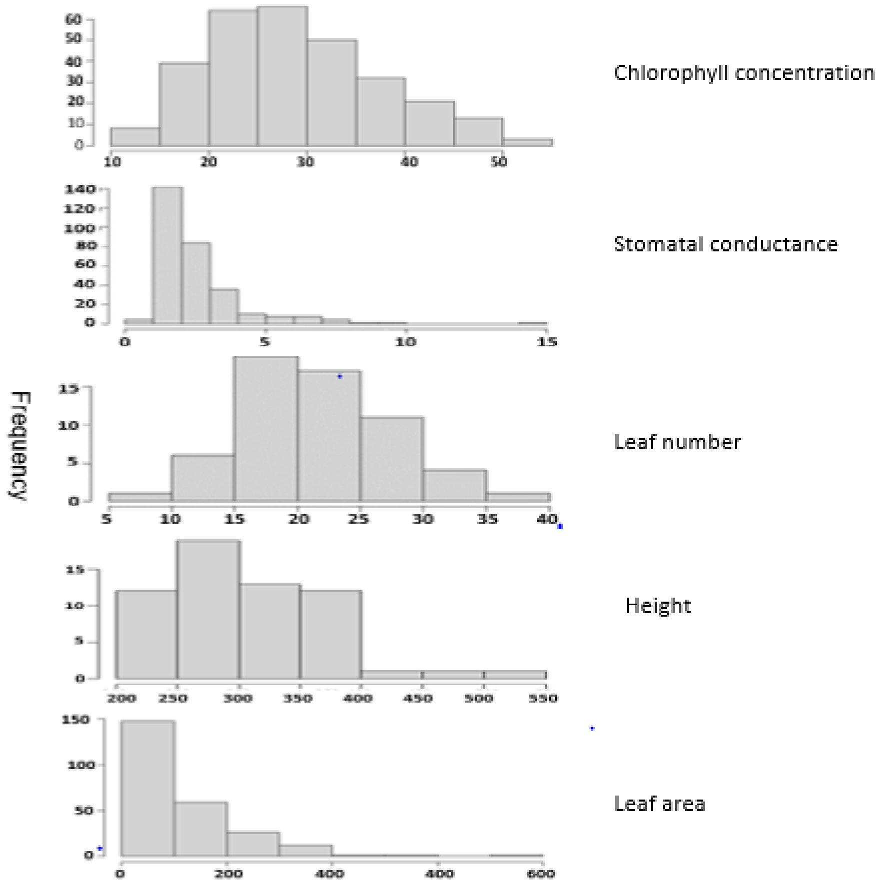

Figure 4.2 Growth and physiological parameter distribution at various spatial configurations.

From Figure 4.2, the central tendence of the data was found between 20 and 30 µmol.m^{-2}, and on Figure 4.1 shows the chlorophyll concentration means for the four different treatments. The difference between the highest and the least chlorophyll concentration mean was 3.78 µmol.m^{-2}. The ANOVA results (Table 4.1), $F_{3,8.7}$ = 30.8, p = 0.0277 which was less than 0.05 significance level, which leads to the conclusion that some of the chlorophyll concentrations of the treatments had significantly different means.

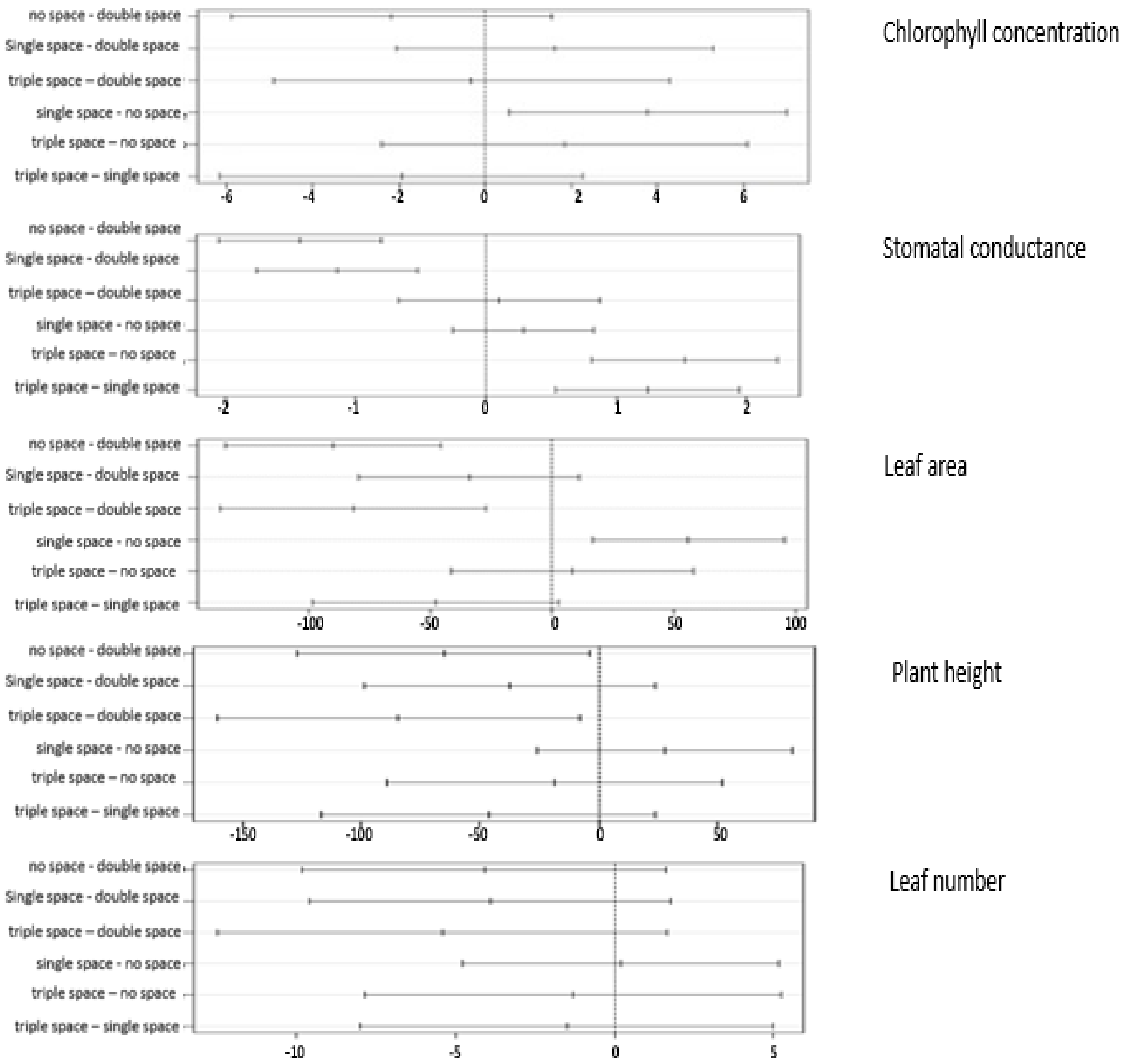

Figure 4.3 Tukey multiple comparisons means of physiological parameters in relation to space, at 95% family-wise confidence level. Mean differences which do not overlap the zero line are statistically significant. No space = 0 cm, single space = 25 cm, double space = 50 cm, triple space = 75 cm.

The Tukey test further revealed that the confidence interval for the difference between the means of single space (25 cm) versus no space (0 cm) range was statistically significant (Figure 4.3) as it did not include zero. However, the confidence intervals for no space versus double space, single space versus double space, triple space versus double space, triple space versus no space and triple space versus single space were not statistically significant because they overlapped zero.

4.2 Stomatal conductance response to varying spatial arrangements

Results in Table 4.2 show that variations in spatial configuration influenced stomatal conductance as well. Stomatal conductance ranged from 1.9 $\pm$ 0.8 to 3.4 $\pm$ 1.6 mmol $m^{-2}s^{-1}$, with the highest stomatal conductance obtained at 75 cm spacing of 3.4 $\pm$ 1.6 mmol $m^{-2}s^{-1}$ and the least at 0 cm spacing 1.9 $\pm$ 0.8 mmol $m^{-2}s^{-1}$. The 25 cm and 50 cm spatial treatments reported 2.2 $\pm$ 1.7 and 3.3 $\pm$ 1.7 mmol m^{-2} s^{-1}, respectively. The distribution of stomatal conductance (Figure 4.2) shows that the central tendence was between 1 and 2 mmol $m^{-2}s^{-1}$. The maximum and minimum stomatal conductance mean values had a difference of 1.52 mmol m^{-2} s^{-1}. The mean stomatal conductance for 0 cm and 25 cm spacing had a slight difference, same applied with means for 50 cm and 75 cm spacing (Figure 4.1). The ANOVA results (Table 4.1) show that $F_{3,1.46} = 18.55$, p = 4.87E-11, which was much less than the 0.05 significance level, leading to a conclusion that some of the stomatal conductance had different means. The Tukey results (Figure 4.3) confirm that the confidence intervals of the differences between the means of no space (0 cm) versus double space (50 cm), single space (25 cm) versus double space (50 cm), triple space (75 cm) versus no space (0 cm) and triple space (75 cm) versus single space (25 cm) ranges were statistically significant and did not include zero. The confidence intervals for mean pairs of triple space versus double space and single space versus no space were not statistically significant since they included zero.

4.3 Leaf number/plant response to spacing

The effects of different spatial configurations on leaf number are presented in Table 4.2. The spacing which obtained the highest number of leaves per plant was 50 cm with 25.4 ± 5.2 leaves, whereas the least number of leaves at 20 ± 3.6 was recorded under 75 cm spacing. The 0 cm and 25 cm spacing attained 21.3 ± 6.1 and 21.5 ± 6.6, respectively. The peak and the least leaf number means had a difference of 5.4 leaves. Leaf number central tendence was between 15 and 25 leaves per plant (Figure 4.2). It was observed in Figure 4.1 that 0 cm spacing, 25 cm spacing and 75 cm spacing had a slight difference in the number of leaves. The ANOVA results revealed that $F_{3,1.46} = 1.8$, $p = 0.157$ (Table 4.1) which was greater than 0.05 significance level, leading to the conclusion that leaf number did not differ between treatments. Data from the Tukey post hoc test confirm that all the mean differences were not statistically significant and the confidence intervals for the differences between all ranges included zero (Figure 4.3).

4.4 Response of plant height to different spatial arrangements

Variations in spatial arrangements influenced the height of pot-grown spinach plants (Table 4.1). Plant height ranged from 264 to 348.2 mm. The 50 cm spacing produced the tallest plants of 348.2 ± 40.9 mm, whereas 75 cm spacing attained the shortest plant height of 264 ± 40.8 mm. 0cm spacing and 25 cm spacing obtained 2842.8 ±

76.6 and 310.5 ± 66.7 mm, respectively. The central tendency was between 250 and 300 mm (Figure 4.2), while the difference between the tallest and shortest plants was 84.17 mm. The 0 cm spacing and 25 cm produced slightly different heights (Figure 4.1). The findings of ANOVA (Table 4.1) show that $F_{3,55} = 3.79$, $p = 0.0153$ which means the heights were significantly different The Tukey post-hoc (HSD) test results showed that the confidence intervals for the differences between the means of no space versus double space and triple space versus double space were statistically significant. However, confidence intervals for single space versus double space, single space versus no space, triple space versus no space, triple space versus single space pairs were not statistically significant and all their paired comparisons included zero (Figure 4.3).

4.5 Leaf Area Response to Various Spatial Configurations

Leaf area responses to variations in spatial configurations of spinach are shown in Table 4.2. The highest leaf area of 169 ± 139 cm^2 was attained at the 50 cm spacing, followed by 25 cm spacing which recorded 135.6 ± 102 cm^2. The smallest leaf area of 79.5 ± 57 cm^2 was attained at 0 cm spacing followed by 75 cm spacing which had 87.8 ± 86 cm^2. The central tendency for leaf area was between 0 and 100 cm^2 (Figure 4.3) and there was a slight difference between leaf areas attained at 0 cm spacing and 75 cm (Table 4.2). ANOVA results in Table 4.1 evinced that $F_{3,96.8} = 11.3$, $p = 5.9E07$ which was less than the 0.05 significance level, meaning that some of the leaf areas had different means. From the Tukey HSD results, the confidence intervals for no space (0 cm) versus double space (50 cm), triple space (75 cm) versus double space (50 cm) and single space (25 cm) versus no space (0 cm) were statistically significant (Figure 4.3) they did not overlap the zero line. The confidence intervals for single space (25 cm) versus double space (50 cm), triple space (75 cm) versus no space (0 cm) and triple space (75 cm) versus single space (25 cm) pairs were however, not statistically significant as they included zero.

4.6 Leaf mass response to different spatial arrangements

Table 4.3 Biomass (g^{-1}) responses of spinach at varying spatial configuration

Treatments (cm)	Leaf mass mean(g^{-1})	Root mass mean (g^{-1})
0	21.28 ± 16	5.79 ± 3.2
25	28.26 ± 25.5	7.18 ± 5.5
50	39.51 ± 21.3	12.63 ± 9.2
75	29.88 ± 14	7.63 ± 3.3
	NS	S

Means with S letter are significant at p = 0.05 and means with NS are non-significant at p = 0.05

Figure 4.4 Above and belowground biomass and space relationships

Leaf mass was not significantly affected by the variations in spatial arrangements (Table 4.1 and 4.3). It ranged from 21.28 ± 16 to 39.51 ± 21.3 g^{-1}, with the highest leaf mass of 39.51 ± 21.3 attained at 50 cm spacing, while the least was with no spacing. Spatial arrangements of 25 cm spacing and 75 cm spacing attained 28.26 ± 25.5 and 29.88 ± 14 g^{-1}, respectively. The difference between the highest and the lowest leaf mass means recorded was 18.23 g^{-1}.

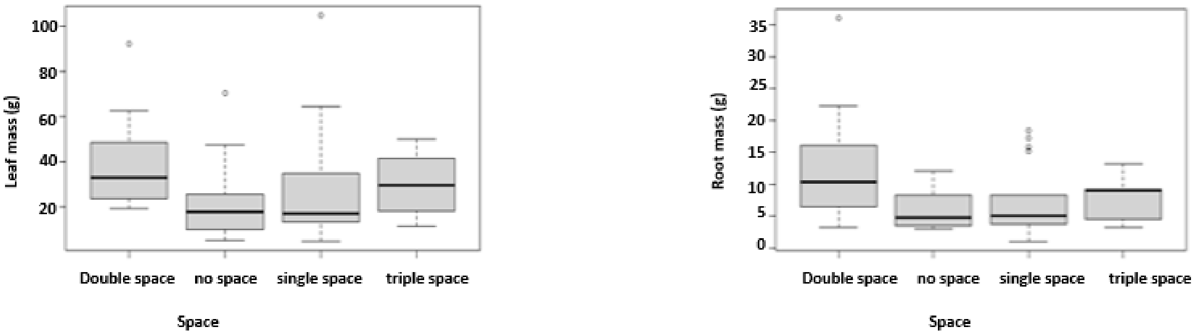

Figure 4.5 Graphical presentation of yield at varying spatial configurations.

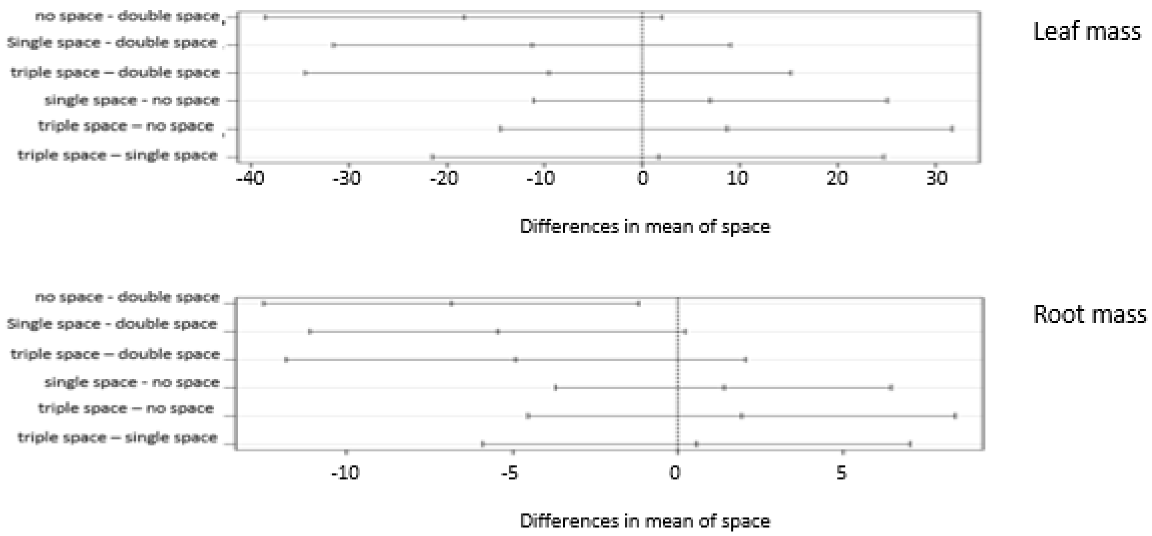

Figure 4.6 Dry mass mean differences in response to space at 95% family wise confidence level. Mean differences which do not overlap the zero line are statistically significant. No space = 0 cm, single space = 25 cm, double space = 50 cm, triple space = 75 cm.

The results in figure 4.5 show that there was a slight difference between leaf mass at no space (0 cm) and single space (25 cm). Leaf mass differences for double space

(50 cm) and triple spacing (75 cm) is visible in Figure 4.4. The ANOVA results (Table 4.2) show that F 3,20.46 = 1.9 p = 0,1382 which means that none of the leaf mass means in the treatments was statistically significant. The Tukey HSD results in Figure 4.6 emphasise this as well

4.7 Root mass response to different spatial arrangements

Results in Table 4.3 suggest that root mass was significantly affected by the variations in spatial configurations. Root mass ranged from 5.79 $\pm$ 3.2 to 12.63 $\pm$ 9.2 g^{-1}, with the most roots recorded at 50 cm spacing while the least grew where there was no space. The 25 cm spacing obtained 7.18 $\pm$ 5.5 g^{-1}, while 75 cm spacing attained 7.63 $\pm$ 3.3 g^{-1} (Table 4.3). The difference between the highest and the least root mass means was 6.84 g^{-1}. All treatments attained different root masses despite 25 cm spacing and 75 cm spacing having a slight difference as presented in Figure 4.4. From the ANOVA results (Table 4.2) $F_{3, 5.72} = 3644$, p = 0.0184 which was significant. Conclusion drawn was that some of the root masses had different means. The Tukey HSD test results showed that confidence intervals for differences between the means of no space versus double space range were statistically significant. By contrast, the confidence intervals for single space versus double space, triple space versus double space, single space versus no space, triple space versus no space and triple space versus single space all were not statistically significant (Figure 4.6).

4.8 Correlation between physiology and yield

Plant growth and physiological parameters responded differently to the variations in spatial configurations of pots. The strengths of the relationships between parameters varied from being positive, negative to not having a relationship at all.

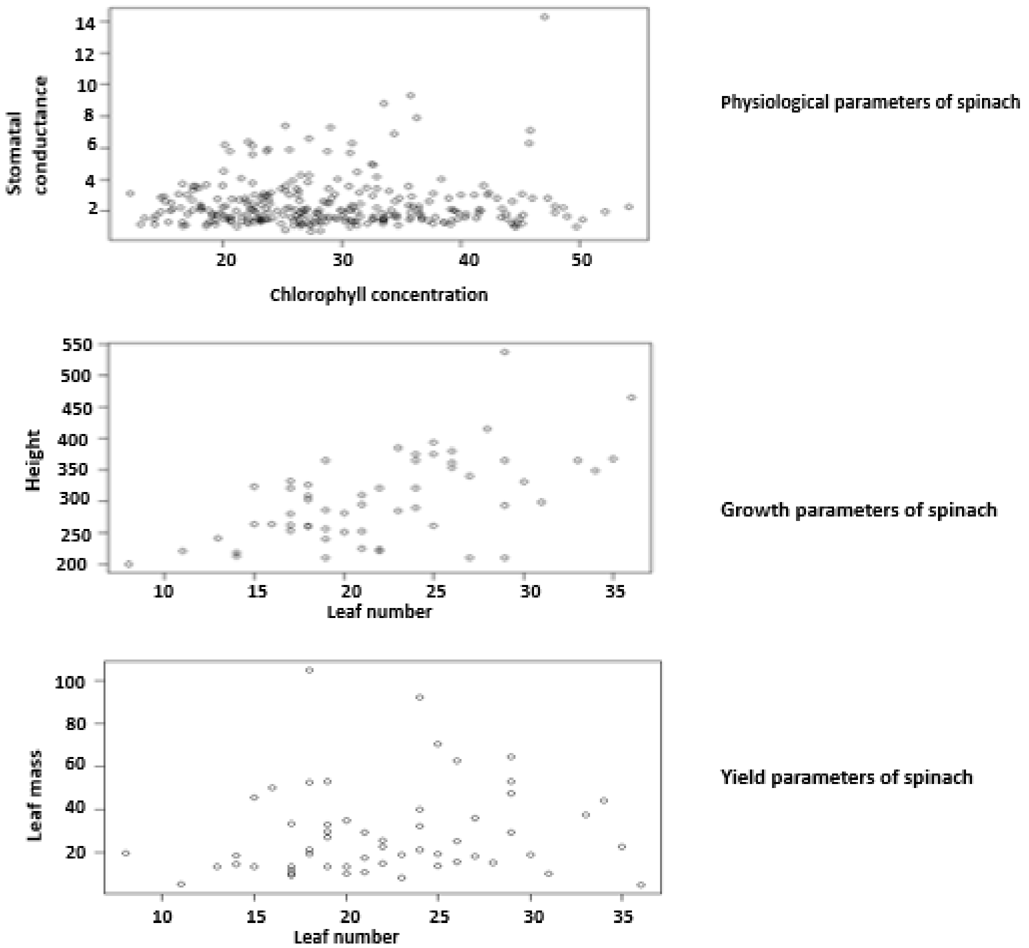

Figure 4.7 Correlation of growth and physiological parameters

4.8.1 Chlorophyll concentration and stomatal conductance

From the analysed results, it was observed that chlorophyll concentration and stomatal concentration did not have a significant relationship. Pearson correlation results show that t = 0.91369, DF = 294, p = 0.3616 and r = 0.05, the p-value was greater than 0.05 significance level, meaning the correlation was not statistically significant. Study results demonstrated that there was very little, to no relationship between chlorophyll concentration and stomatal conductance and the correlation coefficient was strong negative and close to zero. The two parameters did not have a linear relationship, they had no effect on each other. An increase or decrease in one parameter did not have any effect on the other (Figure 4.7).

4.8.2 Stomatal conductance and leaf number

The study results demonstrated that stomatal conductance and leaf number did not have a strong association. $t = 2.0539$, $DF = 57$, $p = 0.04458$ and $r = 0.2625035$, p-value was less than 0.05 significant level. The relationship between the two parameters was not strong. The results show that r was close to zero, meaning that stomatal conductance and leaf number had not much influence on each other. Any increase in one parameter, did not have influence on the other parameter.

4.8.3 Leaf number and plant height

Regarding the relationship between leaf number and plant height, the study results illustrated that leaf number and plant height had a significant association. The results show that $t = 5.4498$, $DF = 57$, $p = 1.123E-06$ and $r = 0.5852921$. p-value was less than 0.05 significance level, which indicated that there was a strong positive relationship between leaf number and height because the correlation coefficient was above 0.5. Moreover, Figure 4.7 results indicated that both parameters influenced each other, an increase in leaf number led to an increase in plant height.

4.8.4 Chlorophyll and plant height

For chlorophyll and plant height, results evinced that the relationship between chlorophyll and plant height was not statistically significant. The Pearson correlation test shows that $t = 1.8755$, $DF = 57$, $p = 0.06586$ and $r = 0.2411$. The p-value was greater than the significant level 0.05 and the correlation was very close to zero. An increase in one parameter had no influence in the other parameters and vice versa.

4.8.5 Leaf number and leaf mass

The results illustrated that there was a significant relationship between leaf number and leaf mass. Results showed that $t = 3.0062$, $DF = 54$, $p = 0.0040$ and $r = 0.3786$. The p-value was less than 0.05 significant level. The results indicated that r was close to zero, meaning to say there was a weak association between the two parameters, as one parameter increased or decreased had no influenced on the other (Figure 4.7).

Chapter 5: Discussion

Efforts to solve the problem of hunger and poverty is causing a fast deterioration to cultivation lands, as the agriculture sector is forced to produce more than its capacity (Fedoroff and Cohen, 1998). Zaman (2011) indicated that there is a reduction in arable lands through urbanization and an increasing land degradation due to agriculture practices and climatic changes. Human activities on another hand are hazardous to the environment and for plant production. They cause both land and air pollution, poor drainage, deforestation, salinity and erosion. If these practices are not well managed, they lead to desertification (Rahman and Debnath, 2015). Human activities also influence the climate by causing unpredictable seasons (Trenberth, 2018) and consequences are more expensive to replenish as they require more resources.

This study assessed the effects of different spatial configurations on pot-grown spinach under field conditions. Previous studies have evaluated space effects on the growth, development and yield of field grown crops such as wheat, maize, Swiss-chard, okra and onions (Cheng et al. 2010; Abuzar et al. 2011; Maboko and Du Plooy, 2013; Maurya et al. 2013; Kahsay et al. 2014). To the best of our knowledge, there is a scant information on the effect of various spatial configurations on the growth, development and yield on the open space pot-grown spinach. Therefore, the findings of this study serve as a benchmark.

From the analysis of the results, it was observed that variations in spatial arrangements significantly affected plant morphological, physiological and yield parameters of pot-grown spinach. Plant adaptation to fluctuations in light capture due to spatial variations affected the chemical reactions (rate of photosynthesis and the exchange of gasses), plant pigment (chlorophyll) as stated in (Nemadodzi, 2015). This also affected the anatomy of the plants (shape, size and structure of plant) of plants which were deprived of light, they had a poor growth and performance to those which were exposed to light, as stated by (Heitholt and Sasserath-Cole, 2010).

Plant height was significantly influenced by the variations in spatial configurations. There was an increasing trend as spacing widened, which encouraged exhibition of the tallest plants at 50 cm spacing. However, there was height inhibition as spacing increased further to 75 cm (Figure 4.2). Disparities in plant height might have been induced by the presence of intraspecific competition, allelopathy and allelobiosis, due to plant competition (for light and gasses). Production of shorter plants at close spacing (0 cm) and widest spacing (75 cm) correlated with the findings of Moniruzzaman (2006) and Sikder et al. (2010), who reported short plants at the close quarters and tall plants as distances increased. Vigorous growth at wider spacing might be attributed to free air circulation and penetration of photosynthetic active radiation (PAR), due to lack of competition. Production of short plants at close encounters might be attributed to the inability of the plants to perform important life processes solely because of the insufficiency of light.

Despite spacing not being statistically significant on leaf number, the different treatments had some variations. The number of leaves increased as spaces widened, despite the widest spacing (75 cm) obtaining the least number of leaves. Optimum plant spacing (50 cm) produced the highest number of leaves. However leaf number decreased when plants were packed tightly (Figure 4.2), this could be an indication of plant-plant interactions through VOC and competition for light. These findings aligned with those of Hasan et al, (2017) and Gashaw and Haile, (2020) who recorded the least leaf number on *Basella Alba* at wider spacing. As spacing became smaller plants reached a point where they started to compete for light, carbon dioxide and the lower leaves discoloured and died due to the intensity of competition. Resource competition drops as space increased and there was allowance for vertical and horizontal expansion of leaves at optimum spacing (50 cm) that led to maximum leaf production, over close spacing.

Spacing had a significant effect on stomatal conductance, it increased with rising space possibly because of more air circulation, more exposure to light and the absence of negative allelochemical interactions between plants. These findings are in line with those of Urban et al. (2017) who observed an increase in stomatal conductance as temperature rose. The ability of a plant to capture light determines its ability to respire (generates carbon dioxide and oxygen), transpire (loose water through evaporation) and photosynthesize (transfer light energy into chemical energy). It is suggested that stomatal opening and closing movements are subject to light absorbed and has the ability to prevent leaf desiccation yet allowing free movement of carbon dioxide (CO_2). Stomata opening is promoted in plants exposed to light and inhibited in plants under low irradiance (Kim et al. 2004). Scarth, (1932) stated that stomatal opening is also encouraged under low CO_2 concentrations, whereas high CO_2 concentrations stimulates the closure of stomata even under bright light leading to plant failure to release oxygen from the leaves encouraging continuous build up. Exhibition of least stomatal conductance by closely spaced plants was probably due to inability of plants to capture enough light, thereby exposing plants to intraspecific competition, which possibly triggered negative interactions through volatile organic compounds. On another hand, the exposure of plants to extreme quanta of light at the widest spacing prevented stomatal closure, thereby inhibiting the enhancement of photosynthesis.

The findings here show that spacing had a significant effect on chlorophyll concentration as well. The highest chlorophyll concentration of 30.6 $\pm$ 8.6 µmol.m^{-2} was observed at closer spacing (25 cm) but decreased under too close spacing (0 cm) to 26.9 $\pm$ 9.7 µmol.m^{-2} (Table 4.2) This was probably due to the inability of plants to capture enough light for elective photosynthesis. Variations in chlorophyll might have been reflecting the intensity of plant-plant interactions and the severity of competition for light. Plant pigments were affected by the changes in light captions due to plants overshadowing each other as they competed for light (Percy and Sim, 1994). This study highlighted that plant adaptation to insufficient light affects plant physiological processes like enzyme reactions, pigments and plant anatomy.

Spacing had a significant effect on leaf area, it enhanced the production of maximum leaf area of 169 $\pm$139 cm^2 at 50 cm treatment, followed by 135.6 $\pm$ 102 cm^2 at 25 cm spacing, 87.8 + 86 cm^2 at 75 cm spacing and the least leaf area of 79.5 $\pm$ 57 cm^2 was obtained at 0 cm spacing (Table 4.2). Production of small leaves at close spacing was probably due to intraspecific competition, as plants competed for light, inhibiting each other's growth. These findings were on par with those of Mondal and Puteh (2013) and those of Streck et al. (2014), which both moled larger leaf areas in wider spacing and the least leaf areas under close spacing.

Plant biomass response to variations in spacing showed an increasing trend as the spacing increased. Dry leaf mass was not statistically significant, whereas dry root mass was statistically significant. The highest dry leaf mass of 39.51 $\pm$ 21.3 g^{-1}

and highest root dry mass of 12.63 $\pm$ 9.2 g^{-1} were obtained at 50 cm spacing, while the least dry leaf mass of 21.28 $\pm$ 16 g^{-1} and the least root mass of 5.79 $\pm$ 3.2 g^{-1} were obtained at close spacing (0 cm) (Table 4.2). Plants in wider spacing obtained large amounts of light which enhanced photosynthetic processes thereby promoting both above and belowground yields. These findings agreed with those of Islam et al. (2014), Streck et al. (2014) and Gashaw and Hail, (2020) who recorded higher dry mass at wider spacings.

From the results it is evident that there was a correlation between leaf number and plant height. An increase in one parameter led to the increase of another (Figure 4.7). However, all other parameters did not have any significant effects on each other. These findings corresponded with those of Paul and Foyer, (2001), who reported that as plant leaves increase, the rate of photosynthesis increases as well. An increase in photosynthesis enables CO_2 intake and light absorption, thereby promoting height increase.

It is evident from the results of the study that space allocation in plant production is an essential practice. It enables plants to acquire important resources which are necessary for growth, development and yield production. Improved production methods, empowers subsistence farmers to produce more, improving the food security of a nation, reducing hunger and poverty as well as promoting health bodies.

5.1 Conclusions

According to data, spacing of container-grown vegetables has a great influence in both the performance and the yield of the plants. Too close spacing compromises both the quality and the quantity of the produce, due to competition for resources. It also reduces photosynthetic active radiation, inhibiting plant growth. This shows that proper space allocation is crucial for crop growth and development and for yield establishment.

It is evident from the results that 50 cm spacing was best for container-grown spinach compared to other spacings used in this study. This spacing managed to produce tall plants with more, broad leaves with a good colour pigment and long roots as well. These were enabled by the absence of resource competition for light and the ability of air to circulate properly. Under close spacing the parameters (plant height, leaf number, leaf area, chlorophyll, conductance and root length) were significantly low due to resource competition. Plants grown under widest spacing (75 cm) had no, to less interaction with other crops and their performance was not significant.

This study therefore suggests that there is a need for farmers to adopt innovative production measures, which are less affected by the shortage of land, climatic conditions, land degradation or any kind of disasters. The aim should be to fully utilise the available land, improve food security and create employment, thereby reducing hunger and poverty, especially in developing countries.

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APPENDIX I: Correlation of parameters

Chlorophyl conductance and stomatal conductance

Pearson's product-moment correlation

data: parameters$chl and parameters$con

t = 0.91369, df = 294, p-value = 0.3616

alternative hypothesis: true correlation is not equal to 0

95 percent confidence interval:

-0.0611636 0.1662082

sample estimates:

cor

0.05321197

Chlorophyll concentration and leaf number

> cor.test(parameters$chl,parameters$no..leafs)

Pearson's product-moment correlation

data: parameters$chl and parameters$no..leafs

t = 0.6935, df = 57, p-value = 0.4908

alternative hypothesis: true correlation is not equal to 0

95 percent confidence interval:

-0.1685597 0.3395984

sample estimates:

cor

0.09147091

Stomatal conductance and plant height

```
> cor.test(parameters$con,parameters$height)
```

Pearson's product-moment correlation

data: parameters$con and parameters$height

t = 0.47085, df = 57, p-value = 0.6395

alternative hypothesis: true correlation is not equal to 0

95 percent confidence interval:

-0.1969779 0.3133320

sample estimates:

cor

0.06224417

Number of leaves and plant height

```
> cor.test(parameters$no..leafs,parameters$height)
```

Pearson's product-moment correlation

data: parameters$no..leafs and parameters$height

t = 5.4498, df = 57, p-value = 1.123e-06

alternative hypothesis: true correlation is not equal to 0

95 percent confidence interval:

0.3872524 0.7317044

sample estimates:

cor

0.5852921

Number of leaves and leaf mass

> cor.test(parameters$no..leafs,parameters$leaf.mass)

Pearson's product-moment correlation

data: parameters$no..leafs and parameters$leaf.mass

t = 3.0062, df = 54, p-value = 0.004008

alternative hypothesis: true correlation is not equal to 0

95 percent confidence interval:

0.1285319 0.5834585

sample estimates:

cor

0.3786379

Number of leaves and root mass

> cor.test(parameters$no..leafs,parameters$rootmass)

> cor.test(parameters$no..leafs,parameters$root.mass)

Pearson's product-moment correlation

data: parameters$no..leafs and parameters$root.mass

t = 2.6202, df = 54, p-value = 0.01139

alternative hypothesis: true correlation is not equal to 0

95 percent confidence interval:

0.08002324 0.55017918

sample estimates:

cor

0.3358579

Number of leaves and leaf area

> cor.test(parameters$no..leafs,parameters$area..cm.2.)

Pearson's product-moment correlation

data: parameters$no..leafs and parameters$area..cm.2.

t = 0.28021, df = 47, p-value = 0.7805

alternative hypothesis: true correlation is not equal to 0

95 percent confidence interval:

 -0.2431505 0.3183788

sample estimates:

cor

0.04083831

Stomatal conductance and leaf area

> cor.test (parameters$con, parameters$area..cm.2.)

Pearson's product-moment correlation

data: parameters$con and parameters$area.cm.2.

t = 0.57418, df = 246, p-value = 0.5664

alternative hypothesis: true correlation is not equal to 0

95 percent confidence interval:

 -0.08838622 0.16041979

sample estimates:

cor

0.0365837

Stomatal conductance and root mass

> cor.test(parameters$con, parameters$root.mass)

Pearson's product-moment correlation

data: parameters$con and parameters$root. mass

t = -0.6434, df = 54, p-value = 0.5227

alternative hypothesis: true correlation is not equal to 0

95 percent confidence interval:

 -0.3422733 0.1798022

sample estimates:

       cor

-0.0872213

Stomatal conductance and plant height

> cor.test(parameters$con,parameters$height)

Pearson's product-moment correlation

data: parameters$con and parameters$height

t = 0.47085, df = 57, p-value = 0.6395

alternative hypothesis: true correlation is not equal to 0

95 percent confidence interval:

 -0.1969779 0.3133320

sample estimates:

       cor

0.06224417

Chlorophyll concentration and plant height

> cor.test(parameters$chl,parameters$height)

Pearson's product-moment correlation

data: parameters$chl and parameters$height

t = 1.8755, df = 57, p-value = 0.06586

alternative hypothesis: true correlation is not equal to 0

95 percent confidence interval:

-0.01598674 0.46825608

sample estimates:

cor

0.2410825

Stomatal conductance and leaf number

> cor.test(parameters$con,parameters$no..leafs)

Pearson's product-moment correlation

data: parameters$con and parameters$no..leafs

t = 2.0539, df = 57, p-value = 0.04458

alternative hypothesis: true correlation is not equal to 0

95 percent confidence interval:

0.006884028 0.485920992

sample estimates:

cor

0.2625035

Stomatal conductance and leaf mass

> cor.test(parameters$con,parameters$leaf.mass)

Pearson's product-moment correlation

data: parameters$con and parameters$leaf.mass

t = -0.79929, df = 54, p-value = 0.4276

alternative hypothesis: true correlation is not equal to 0

95 percent confidence interval:

-0.3607759 0.1592976

sample estimates:

cor

-0.1081314

APPENDIX II: Ethical Approval

UNISA university of south africa

UNISA-CAES HEALTH RESEARCH ETHICS COMMITTEE

Date: 15/02/2021

Dear Ms Chikanda

Decision: Ethics Approval from 11/02/2021 to 31/01/2024

NHREC Registration # : REC-170616-051
REC Reference # : 2021/CAES_HREC/012
Name : Ms P Chikanda
Student #: 55627919

Researcher(s): Ms P Chikanda
chikandapopular@gmail.com

Supervisor (s): Mr M Maluleke
malulm@unisa.ac.za; 011-471-3838

Dr L Khomo
khomolm@unisa.ac.za; 011-471-2956

Working title of research:

Above ground effects of spacing on growth, performance and yield of spinach

Qualification: MSc Agriculture

Thank you for the application for research ethics clearance by the Unisa-CAES Health Research Ethics Committee for the above mentioned research. Ethics approval is granted for three years, **subject to submission of yearly progress reports. Failure to submit the progress report will lead to withdrawal of the ethics clearance until the report has been submitted.**

The researcher is cautioned to adhere to the Unisa protocols for research during Covid-19.

Due date for progress report: 31 January 2022

Please note the points below for further action:

University of South Africa
Preller Street, Muckleneuk Ridge, City of Tshwane
PO Box 392 UNISA 0003 South Africa
Telephone: +27 12 429 3111 Facsimile: +27 12 429 4150
www.unisa.ac.za

1. More detail is required on the statistical analysis: What is the motivation for using Anova? The researcher should specify the model and the variables that will be applied.

*The **minimal risk application** was **reviewed** by the UNISA-CAES Health Research Ethics Committee on 11 February 2021 in compliance with the Unisa Policy on Research Ethics and the Standard Operating Procedure on Research Ethics Risk Assessment.*

The proposed research may now commence with the provisions that:

1. The researcher will ensure that the research project adheres to the relevant guidelines set out in the Unisa Covid-19 position statement on research ethics attached.
2. The researcher(s) will ensure that the research project adheres to the values and principles expressed in the UNISA Policy on Research Ethics.
3. Any adverse circumstance arising in the undertaking of the research project that is relevant to the ethicality of the study should be communicated in writing to the Committee.
4. The researcher(s) will conduct the study according to the methods and procedures set out in the approved application.
5. Any changes that can affect the study-related risks for the research participants, particularly in terms of assurances made with regards to the protection of participants' privacy and the confidentiality of the data, should be reported to the Committee in writing, accompanied by a progress report.
6. The researcher will ensure that the research project adheres to any applicable national legislation, professional codes of conduct, institutional guidelines and scientific standards relevant to the specific field of study. Adherence to the following South African legislation is important, if applicable: Protection of Personal Information Act, no 4 of 2013; Children's act no 38 of 2005 and the National Health Act, no 61 of 2003.
7. Only de-identified research data may be used for secondary research purposes in future on condition that the research objectives are similar to those of the original research. Secondary use of identifiable human research data require additional ethics clearance.
8. No field work activities may continue after the expiry date. Submission of a completed research ethics progress report will constitute an application for renewal of Ethics Research Committee approval.

Note:

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*The reference number **2021/CAES_HREC/012** should be clearly indicated on all forms of communication with the intended research participants, as well as with the Committee.*

Yours sincerely,

Prof MA Antwi
Chair of UNISA-CAES Health REC
E-mail: antwima@unisa.ac.za
Tel: (011) 670-9391

Prof SR Magano
Acting Executive Dean : CAES
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APPENDIX III: Turnitin report

Turnitin Originality Report

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MSc Agriculture dissertation By P Chikanda

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