GROWTH AND DEVELOPMENT OF BABY SPINACH (*Spinacia oleracea* L.) WITH REFERENCE TO MINERAL NUTRITION

by

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DECLARATION

I declare that **Growth and development of baby spinach (*Spinacia oleracea* L.) with reference to mineral nutrition** is my own work and that all the sources that I have used or quoted have been indicated and acknowledged by means of complete references. The thesis was subjected to submission as per University policy.

Signed …… Date: …….

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ABSTRACT

Baby spinach is a relatively new crop in South Africa with both commercial significance and reputed health benefits. It is known to assist in combating degenerative conditions associated with ageing, such as heart disease, cardiovascular disease, Alzheimer's disease, cataracts and several forms of cancer. Three parallel NPK trials were conducted to investigate the effects of nitrogen (N), phosphorus (P) and potassium (K) on the growth and development of baby spinach. N and P treatments were arranged as (0, 45, 75, 105, 120 kg.ha $^{-1}$ N and P), and K treatments were arranged as (0, 63, 85, 127, 148 kg.ha $^{-1}$) in a randomised complete block designed with four replicates. Results showed that yield, dry matter, chlorophyll content and Leaf Area Index (LAI) were significantly increased by increasing the N application, while K had a significant effect on the LAI but not on yield, dry matter, chlorophyll content or stomatal conductance. Nitrogen treatments quadrupled fresh yield, dry matter and chlorophyll content, reaching maximum impact at 75 kg.ha^{-1} N. Phosphorus application showed significantly increased yields, dry matter and chlorophyll content, reaching maximum impact at 75 kg.ha^{-1} P. Therefore, to achieve optimum growth with N and P, 75 kg.ha^{-1} is recommended. The optimum rates of N, P and K were then used to formulate a NPK combined trial which was arranged as 0, 30:30:40, 45:45:60, 60:60:70, 75:75:90 kg.ha^{-1} in a randomised complete block design with three replicates. The results showed that maximum impact on yield, chlorophyll content, fresh and dry matter was achieved when combined NPK was applied at 45:45:60.

Key words: *Baby spinach, biomass, chlorophyll content, dry matter, mineral nutrition, nitrate, stomatal conductance.*

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CHAPTER 1

Introduction

1.1 Background

Baby spinach (*Spinacia oleracea* L.) belongs to the family Brassicaceae and is an annual cool season, green leafy vegetable which is eaten raw (Conte *et al*. 2008). Spinach is thought to be native to Southwest Asia where it was first cultivated by the Persians. It is now cultivated throughout the world, with the exception of the tropics (USDA, 2005). Depending on the cultivar, the leaves are small (usually no longer than 6 cm), sweet tender and smoother than semi-savoy or savoy spinach (Bergquist *et al*. 2005). Like other leafy greens, baby spinach has a high water content and is harvested 35 days after planting (DAP) instead of the 40 days or more required for normal spinach (Rico *et al*. 2007).

Baby spinach is more expensive than normal spinach and is sold loose rather than in bunches. It is often used in salads because of its attractive flavour and delicate texture. The stalks can also be eaten and should not be wasted (Bergquist *et al*. 2005).

Spinach is a long-day plant that is prone to bolting during summer (Bergquist *et al*. 2005) but this is not often a problem in the case of baby spinach as the leaves are harvested early (Will *et al*. 1998).

High fruit and vegetable intake is known to have a positive impact on human health and is linked to decreased risk of most of the degenerative diseases associated with ageing, such as cardiovascular disease (Christen, 2000; Manach *et al*. 2005; Khaw *et al*. 2001; and Arts & Hollman, 2005) Alzheimer's disease (Commenges *et al*. 2000), cataracts (Brown *et al*. 1999), and several forms of cancer (Williamson, 1996; Liu *et al.* 2000; Gandini *et al.* 2000; Liu *et al*. 2001; Joshipura *et al*. 2001; & Kang *et al.* 2005).

The consumption of baby leaf vegetables such as rocket, lamb's lettuce, headless lettuce and spinach is increasing, and they are particularly in demand for mixed salads, both as fresh market products and ready-to-use vegetables (Bergquist, 2006). The nutritional quality of vegetables can be affected by many pre- and post-

harvest factors (Kader, 2008). Fertilisation is one of the most practical and effective pre-harvest agronomic practices to improve the yield and nutritional quality of crops for human consumption (Arts & Hollman, 2005).

Baby spinach is a relatively new crop in South Africa where it is becoming increasingly popular. It is known to be a healthy vegetable with relatively high levels of bioactive compounds (Gil *et al.* 1999) such as vitamin C, vitamin A and minerals (USDA, 2005). In addition to the health benefits mentioned above, baby spinach has the advantage of a short culture time and shelf life (Bergquist, 2006), making it an excellent model crop (Ninfali & Bacchioca, 2004). However, there is insufficient data on the effects of agronomic practices, such as providing mineral nutrition, on the growth and development of baby spinach.

The aim of this study was therefore to determine the recommended rates of nitrogen (N), phosphorus (P) and potassium (K) mineral nutrition for optimum growth and development of baby spinach. After optimum levels for N, P and K nutrition were determined, the interactive effects of combined NPK nutrition were investigated.

1.2 Problem statement

Baby spinach is a relatively new crop in South Africa with both commercial significance and reputed health benefits such as reducing the occurrence of various forms of cancer, cholesterol, cardiovascular disease and osteoporosis in human beings. The demand for baby spinach in South Africa currently exceeds supply. To increase yield production, farmers are applying normal spinach fertiliser rates but these are not recommended for baby spinach since they may have a negative effect on both yield and health benefits. Data on the recommended application rates of major nutrients such as N, P and K, and the combination NPK, are not readily available in South Africa.

1.3 Motivation for the study

Vegetables which require minimum processing and are consumed fresh have gained in importance and recognition in the worldwide vegetable market (Brecht *et al*. 2004). The increase in demand for baby leaf vegetables has resulted in many farmers gearing their production towards baby vegetables such as baby lettuce, baby spinach and baby Swiss chard (Vernieri *et al*. 2006). Baby spinach is known to have health benefits due to its relatively high levels of bioactive compounds,

antioxidants and phytochemicals (Gil *et al*. 1999). Its consumption is known to have positive effects on human health and has been linked to decreased risk of most degenerative diseases associated with ageing (Williamson, 1996), such as heart disease (Liu *et al.* 2000), cardiovascular disease (Gandini *et al*. 2000), Alzheimer's disease (Liu *et al*. 2001) cataracts (Joshipura *et al*. 2001), and several forms of cancer (Kang *et al*. 2005).

The production of baby leafy vegetables has been increasing in South Africa. However, there is currently no information available in the country on recommended fertiliser application rates for this crop. It is envisaged that this study will help farmers understand the optimum fertiliser rates for growing baby spinach and will contribute to job creation in alignment with the New Growth Path (NGP) Strategy of the Department of Economic Development of South Africa and the National Development Plan (Department of Economic Development, 2011), since the cultivation and harvesting of baby spinach as well as other vegetables is labour intensive.

1.4 Study aim

The aim of the study was to evaluate the effect of different N, P and K applications on the growth and development of baby spinach, and, once optimum N, P, and K fertiliser rates had been established, to investigate the interactive effect of combined NPK nutrition on the growth and development of the plants.

1.5 Objectives

(1) To determine the effect of applications of N, P and K fertilisers on the growth and development of baby spinach.

(2) To determine the effect of combined NPK on the growth and development of baby spinach.

1.6 Hypotheses

(1) N, P and K fertilisers have no influence on the growth and development of baby spinach.

(2) Combined NPK fertiliser has no effect on the growth and development of baby spinach.

1.7 Ethical considerations

No ethical considerations were discerned in this project. (Appendix 2)

CHAPTER 2

Literature review

2.1 Health benefits of baby spinach

High intake of fruit and vegetables is known to have a positive effect on human health and play a role in alleviating the progressive symptoms of most degenerative diseases associated with ageing, such as heart disease, cardiovascular disease, Alzheimer's disease, cataracts and several forms of cancer (Williamson, 1996; Liu *et al.* 2000; Gandini *et al.* 2000; Liu *et al*. 2001; Joshipura *et al*. 2001; & Kang *et al.* 2005). These benefits have been attributed to the high nutritional value and high concentrations of bioactive compounds (ascorbic acid, flavonoids and carotenoids) found in vegetables, partly due to the antioxidative action of some of these compounds (Bergquist, 2006) as well as minerals and folate (Muller, 1997; & Davey *et al*. 2000).

2.2 Nutritional composition of baby spinach

Baby spinach is high in vitamin K, an essential nutrient that contributes to bone health (Sies, 1997). The major micronutrients in spinach are vitamin A (from beta (β)-carotene) (Bergquist, 2006), vitamin C (Institute of Medicine, 2000), folate (Lester & Crosby, 2002), and minerals such as calcium and iron (Zimmermann & Zentgraft, 2005). Baby spinach plays a role in protecting the mucous membrane of the stomach (Bergquist, 2006) and can therefore also act as an anti-ulcerative (Lee & Kader, 2000).

The presence of minerals, vitamins, pigments, phytonutrients, and minerals such as potassium, manganese, zinc, magnesium, iron and calcium makes baby spinach a healthy crop for human consumption (Toledo *et al*. 2003). It is one of the most nutrient-dense foods in existence, low in calories and high in vitamins (Sies, 1997). One cup of this leafy green vegetable exceeds the daily dietary requirements for vitamin K, vitamin A and protein (Lester & Crosby, 2002).

2.2.1 Phytochemicals

Baby spinach is high in β–carotene, a precursor molecule that can be converted into vitamin A in the body (Edenharder *et al*. 2001). It is an extremely nutritious

vegetable, rich in both core nutrients and phytochemicals such as the carotenoids (van het Hof *et al*. 1999; & Castenmiller *et al*. 1999), β-carotene (ATBC, 1994; and Omenn *et al*. 1996), lutein (Bergquist, 2006), zeaxanthin (Demmig-Adams *et al*. 1996) and phenolic compounds (Borek, 1991). Lutein and zeaxanthin are carotenoids that act as antioxidants in the body and also help reduce the risk of cataracts and age-related macular degeneration (Brown *et al*. 1999). Epo xanthophyllis which is found in the carotenoids in baby spinach protect against prostate cancer (Kopsell *et al*. 2006).

A study in 2006 investigated the correlation between the risk of prostate cancer and the intake of vegetable, including baby spinach, broccoli, cauliflower, cabbage, Brussels sprouts, mustard greens, turnip greens, collards and kale. Of all the vegetables, only baby spinach was found to provide significant protection against aggressive prostate cancer, defined as Stage III or IV prostate cancer with a Gleason score of at least 7 (Kopsell *et al*. 2006; & Asia *et al*. 2004).

Another study revealed that women who consumed baby spinach more than twice a week were less likely to develop breast cancer than women who did not consume any (Longnecker *et al*. 1997).

Studies of the role of normal spinach or spinach extracts in the development of cancer in mice have also shown that the number of induced papillomas in a cancer were reduced through the topical and oral administration of a water-soluble spinach extracts (Nyska *et al*. 2001). Neoxanthin from spinach inhibited the proliferation of prostate cancer cells in a laboratory study (Kopsell *et al*. 2006), and 13 flavonoids from spinach showed anti-mutegenic activity in a bacteria-based model (Asia *et al*. 2004). Folate, tocopherol and chlorophyllin are constituents of spinach which are useful in treating and prevention against a variety of cancers such as bladder, liver and lung cancer (Edenharder *et al*. 2001).

2.2.2 Chlorophyll content

Some research suggests that chlorophyll binds to mutant DNA and prevents it from proliferating, which is an important factor in prevention of cancer, when combined with vitamin C content (Forman & Altman, 2004), chlorophyll may be used as a parameter for fresh-cut vegetable quality evaluation (Ninfali & Bacchiocca, 2004).

2.2.3 Amino acids and coenzymes

Glutathione, an extremely important endogenous antioxidant synthesised within the body, is relatively rare in foods but is found in baby spinach. One of its major functions is to protect DNA from oxidation but it also detoxifies carcinogens, boosts the immune system, supports liver health and reduces inflammation. The enzyme glutathione, which is reductase dependent, is usually reduced back to ascorbic acid and α-lipoic acid which are vital antioxidants largely synthesised in the body but also present in some foods (Davey *et al*. 2000). Glutathione is a cysteine-containing peptide found in most forms of aerobic life. Although it is not a dietary requirement, it is synthesised in the cells from its constituent amino acids (Hamid *et al*. 2010). It is important for energy metabolism and its antioxidant activity may provide protection against chronic diseases. Another endogenous compound, coenzyme Q_{10}, is a critical component in energy metabolism (Demmig-Adams *et al*. 1996) but also acts as an antioxidant in cell membranes and lipoproteins (Lopez-Ayerra *et al*. 1998). The best food sources of protein are meat, fish and oils, but spinach is one of the best vegetable sources (Rico *et al*. 2007). Blood cholesterol may be lowered by consuming D-glucaric acid (Joseph *et al.* 2002).

Due to limited research, betaine is not a well-known compound. It is found in microorganisms, plants and animals and is a significant component of many foods including baby spinach (Sakamoto *et al*. 2002; & Zeisel *et al.* 2003). It is thought that cardiovascular disease may be prevented by lowering the levels of homocystein which is a compound associated with the development of heart disease (Joseph *et al.* 2002). By reducing vascular risk factors, enhancing performance and protecting internal organs, betaine has proven itself to be an important nutrient in the prevention of chronic disease (Stuart & Graig 2004). Tokar & Berg (2002) found that the accumulation of fat in the liver, which affects liver metabolism such that it can contribute to the development of a number of diseases including coronary, cerebral, hepatic, and vascular diseases, may be prevented or reduced by consuming betaine. Studies of healthy and diabetic humans show that a high-fat diet leads to hepatic steatosis (Abdelmalek *et al*. 2001) which can be prevented by the ingestion of beatine (Zhu *et al*. 2003 & Neuschwander- Tetri, 2001).

2.2.4 Carotenoids

Carotenoids are yellow, orange or red pigments found in fruits, flowers and green vegetables which are responsible for colour development (Muller, 1997), although the colour of carotenoids is generally disguised by chlorophyll. Carotenoids are tetraterpenoids, which are built upon a 5-carbon isoprenoid unit, and are hydrophobic (Krinsky & Johnson, 2005). There are two groups of carotenoids, the hydrocarbon carotenes and the oxygenated xanthophylls (Demmig-Adams *et al*. 1996). Carotenes, such as α- and β-carotene and lycopene, are predominantly orange or red-orange pigments, whereas xanthophylls, such as lutein, viloaxanthin, zeaxanthin, antheraxanthin and neoxanthin are primarily yellow (Asplund, 2002). Carotenoids play a role in photosynthesis as accessory pigments absorbing light energy and transforming it into chlorophyll (Demmig-Adams *et al*. 1996).

Carotenoids are easily absorbed in the body if accompanied by a small amount of oil or fat in a meal (Hedges & Lister, 2007). A study conducted with humans demonstrated that serum carotenoid concentrations as well as macular pigment optical density (a possible predictor of macular degeneration) increased with an increase in the consumption or intake of spinach carotenoids (Kopsell *et al*. 2006).

2.2.5 Flavonoids

Flavonoids are compounds with yellow to white or blue, and purple to red colours which are often referred to as polyphenols (Lister, 1999 & Kris-Etherton *et al*. 2002). Baby spinach contains more than a dozen flavonoid compounds with anti-inflammatory and anti-cancerous properties which serve as antioxidants (Greenberg *et al*. 1996 & Kolb *et al*. 2001).

Various factors such as food matrix, human intestinal micro-flora and flavonoid structure determine the bioavailability of flavonoids (Manach *et al.* 2005). Studies conducted on the epidemiology of flavonoids indicated that they lower the risk of coronary heart disease and dementia (Hertog *et al*. 1995 and Knekt *et al*. 1996; Commenges *et al*. 2000 & Neuhouser *et al*. 2004).

Factors such as genotype (Cieslik *et al*. 2006 and Kalt *et al*. 2001), growing conditions (Cao *et al*. 1996), growth stage (Prior *et al*. 1998), postharvest handling (Wang & Zheng 2001) and storage conditions (Kalt *et al*. 1999) determine the level of flavonoid content in plants (Patil *et al.* 1995 & Howard *et al*. 2002). However,

baby spinach contains more than a dozen individual flavonoid compounds, which work together as cancer fighting antioxidants (Houstis *et al*. 2006) by neutralising free radicals in the body. A study conducted in Europe showed fewer cases of breast cancer among women who ate baby spinach regularly (Kris-Etherton *et al*. 2002); (Le Marchad, 2002 & Neuhouser, 2004). Skin and stomach cancers in laboratory animals were reduced by a diet with spinach extracts *(*Fico *et al*. 2000 & Vogt & Gulz, 1994*)*.

2.2.6 Antioxidants

Most of the flavonoid and carotenoid nutrients found in baby spinach that have anti–inflammatory properties, provide antioxidant benefits (Lurie, 2003). Baby spinach is an excellent source of other antioxidant nutrients such as vitamin C, vitamin E, β-carotene, manganese, zinc and selenium, which help reduce the risk of numerous health problems associated with oxidative stress (Lampe, 1999). Human blood vessels are susceptible to damage from oxidative stress, but this may be reduced by consuming baby spinach which contributes to reduced risk of several blood vessel related problems, including atherosclerosis and high blood pressure (Teddy & James, 2008).

A number of studies have shown that baby spinach has strong antioxidant activity and high levels of antioxidant compounds such as phenolics and carotenoids (Fico *et al*. 2000). Ascorbic acid reported in baby spinach is a well-known antioxidant and enzyme co-factor with many roles in human health (Hedges & Lister, 2007).

One of the major health benefits attributed to two major compounds in baby spinach, lutein and zeaxanthin, is that of protection against eye diseases such as macular degeneration and the gradual loss of central vision associated with old age (Teddy & James, 2008). According to research conducted at Oak Ridge National Laboratory in 1993, consumption of baby spinach can lead to regaining two pigments, *inter alia* Retinis pigmentosa, and preventing age-related macular degeneration. Beta-carotene, lutein and xanthenes found in baby spinach are beneficial for eyesight (Bird *et al*. 1995) and prevent vitamin A deficiency disease (Seddon *et al*. 1992), itchy eyes (Bird *et al*. 1995) eye ulcers and dry eyes (Neuhouser, 2004*)*.

Epidemiological and laboratory studies have also shown that normal spinach, spinach extracts and spinach compounds may delay or retard age-related loss of brain function, reduce the extent of post-ischaemic stroke damage to the brain, and protect against cancer through various different mechanisms (Hedges & Lister, 2007).

The study which involved feeding young rats a freeze-dried aqueous spinach extract and observing changes related to mental function as they aged, showed that a diet with spinach extracts retarded age-related decline the most. Although two groups of rats had been fed other diets high in antioxidants (strawberry extracts or vitamin E supplements), the beneficial effects were most pronounced in the spinach-fed group (Joseph *et al*. 1998).

A further study demonstrated that various antioxidants found in baby spinach extracts reversed the age-related decline of neuronal behavioural parameters (Joseph *et al*. 1999).

Wang *et al*. (2005) reported that rats fed a diet supplemented with baby spinach showed reduced symptoms of post-ischaemic stroke and brain damage. Lowered blood pressure was noticed in spontaneously hypertensive rats fed peptides isolated from leaf rubisco, which has been shown to have anti-cholinesterase (ACE) inhibitory properties (Joseph *et al*. 1998).

Spinach has high potassium content and low sodium content (Lampe, 1999). This mineral composition is very beneficial for patients with high blood pressure, as potassium lowers blood pressure while sodium raises it. The folate present in spinach may contribute to the maintenance of proper blood flow, relaxation of blood vessels and reduced levels of hypertension (Yang *et al*. 2003).

2.3 Other health benefits of baby spinach

2.3.1 Skin protection

Various phytonutrients and pigments found in baby spinach have been shown to protect the skin from harmful rays of the sun, including the Ulta-Violet rays. These not only protect the skin but repair damaged genes to some extent, thereby preventing skin cancer in the long run (Hedges & Lister, 2007).

2.3.2 Foetus development

A growing foetus needs the folate found in baby spinach for proper development of the new nervous system. In its absence, the foetus is likely to suffer from defects such as cleft palate or spina bifida (Bergquist *et al*. 2006). Pregnant mothers are advised to consume the vitamin A found in baby spinach in larger quantities in order to enhance lung development in the foetus (Halliwell & Gutteridge, 1989).

2.3.3 Cardiovascular health

Research conducted by Whole Foods indicated that baby spinach is an excellent promoter of cardiovascular health. The antioxidant properties (water-soluble vitamin C and fat-soluble β-carotene) work together to promote good cardiovascular health by preventing the harmful oxidation of cholesterol, which is a danger to the heart and arteries (Halliwell & Gutteridge, 1989). The magnesium in baby spinach reduces blood pressure levels (Reams, 2002).

An anti-oxidant component of baby spinach, factor C0-Q_{10}, plays an important role in strengthening muscles, especially the heart muscle which continuously pumps blood to all parts of the body. The C0-Q_{10} can be useful in the prevention and treatment of many cardiovascular diseases such as hyperlipidemia, heart failure, hypertension and coronary heart diseases *(*Halliwell & Gutteridge, 1989).

2.3.4 Bone development

The vitamin K found in baby spinach functions to retain calcium in the bone matrix, resulting in bone mineralisation. Its role extends beyond supporting blood clotting to include a role in bone metabolism, and it also offers potential protection against osteoporosis (Shearer *et al*. 2004). Minerals such as manganese, copper, magnesium, zinc and phosphorus also help in the building of strong bones, thus helping to osteoporosis (Joseph *et al*. 2002).

2.4 Factors affecting the growth of and nutrients in baby spinach

The amount of core nutrients and other phytochemicals in food is determined by factors such as climatic conditions, the plant variety or cultivar, agronomic issues such as soil type, cultivation protocols such as irrigation, pest control, fertiliser use and maturity at harvest, as well as processing practices such as harvesting, storage and processing methods (Howard *et al*. 2002).

2.4.1 Climatic conditions

Baby spinach is a quick maturing, cool season vegetable crop. Seed germinates at 2° to 30°C, but 7°to 24°C is optimal (Bergquist, 2006). The plant grows in conditions of 5° to 30°C, but growth is accelerated at 15° to 18°C (Meyer & Anderson, 1952). Baby spinach can withstand low temperatures of -9° to -6°C without significant damage (Conte *et al*. 2008). Freezing temperatures do damage small seedlings and young plants, but more mature plants can tolerate sub-freezing temperatures for weeks (Bergquist *et al*. 2005).

2.4.2 Edaphic factors

A variety of soils are used for spinach production, but in most regions sandy loam is preferred (Abdelmalek *et al*. 2001). Baby spinach is particularly sensitive to saturated soil conditions and is also moderately salt sensitive. Spinach grows best in slightly acid to slightly basic soil (pH 6-7.5) but some very successful production occurs in soils with a pH of above 8.0 (Nonnicke, 1989).

2.4.3 Cultivars

In California, the smooth or flat leaf spinach cultivars are grown almost exclusively, although some semi-savoy varieties are also cultivated. All baby spinach cultivars commercially grown in California are hybrids, primarily because resistance to disease and bolting has been bred into the hybrids. Increasing day length, maintaining high temperatures and ensuring adequate fertilisation and irrigation keep bolting to a minimum (Joseph *et al*. 2002). The most common baby spinach cultivars grown in South Africa are Ohio, Virofla, Corvette and Corvairt (Hygrotech Seed Company, South Africa). The leaves are small (usually no longer than 6 cm), sweet tender and smoother than semi-savoy or savoy spinach (Bergquist *et al*. 2005).

2.4.4 Planting

Baby spinach is direct seeded either into the soil or into planting trays. The Californian industry is known for using very high seed planting densities and a large number of seed lines per bed. In general, baby clipped spinach is only planted in 80 inch (203 cm) wide beds. Spinach seed is planted ½ to ¾ inch (1.2 to 1.9 cm) deep, depending on the method of planting and the soil conditions (Patil *et al.* 1995). In California, baby spinach is usually picked 35 days after planting (DAP) as opposed

to the 45 DAP for normal spinach (Bergquist, 2006). In South Africa, there is no data that is used as recommendations for planting baby spinach due to the fact that it is fairly a new crop.

2.4.5 Irrigation methods

Depending on initial soil conditions, 5-10 cm of water is applied by means of sprinklers to moisten the soil for tillage and seedbed preparation. All baby spinach fields in California are sprinkler irrigated to germinate the seed. Two to three irrigations are required between seeding and emergence. Short sprinkler use is recommended every 2 days until emergence to prevent the formation of a soil crust and to replace moisture lost by evaporation (Joseph *et al.* 2002). Many studies have reported that, with several species, water deficiency typically results in depressed plant growth, decreased plant quality and decelerated maturation with low marketable yield (Guichard *et al*. 2005; Ho, 1996b; and Miras-Avalos *et al*. 2013). However, In South Africa, there is no data on irrigation guidelines that is used as recommendations for planting baby spinach due to the fact that it is fairly a new crop.

Baby spinach has a relatively shallow root system and thrives on frequent, short irrigations that maintain a uniformly moist soil for maximum leaf production (Bergquist, 2006). However, care must be taken to avoid saturated conditions as the plants are sensitive to overwatering, particularly in the case of heavy soil textures. Saturated conditions can contribute to soil-borne diseases and to abiotic rotting of the roots, crown, and lower leaves (Bergquist, 2006).

2.4.6 Fertilisation

Baby spinach needs applications of the major fertilisers (N, P and K) at the right time and in the right quantities to deliver the best yield. Fertiliser is a concentrated form of food as compared with bulky conditioners and organic manures. Baby spinach is moderately fertilised, and the fertiliser rate should be determined taking soil type, recent cropping history and soil test results into account (Lucier *et al.* 2004).

2.4.6.1 Nitrogen

Baby spinach is a short season crop that is harvested when the crop is young. As a result, the nutrient uptake is relatively low. For instance, the nitrogen (N) content of baby spinach may vary between 20 and 40 pounds per acre (22 to 45 kg/ha) in Californian soils (Gastal & Lemaire, 2002). Baby spinach is a medium to heavy nitrogen feeder, and since the application of nitrogen encourages leaf growth, it is useful for promoting the growth and development of baby spinach and other leafy vegetables (Bergquist *et al*. 2006). The effects of N fertilisation on vegetable yields have long been recognised and clearly demonstrated (Collins & McCoy, 1997; and Blom-Zandstra, 1989). Nitrogen fertilisation increases foliage which reduces light intensity around the plant, which in turn may affect the concentration of flavonoid compounds (Mozafar, 1994 and Lee & Kader, 2000). Nitrogen is the most abundant element in plants. It plays a role as a constituent of protein, nucleic acids, chlorophyll and growth hormones (Barker *et al*. 1974). Adequate N application is very important for optimising crop yields, but excessive applications may lead to groundwater contamination (Jaynes *et al*. 2001). Nitrogen deficiency is characterised by an immediate decrease in growth rate and a loss of chlorophyll from the leaves so that they become light green progressing to yellow. Continuous N stress may cause the entire plant to appear yellowish-green or yellow (Blom-Zandstra, 1989).

2.4.6.2 Phosphorus

Phosphorus (P) applications should be determined on the basis of soil test results for bicarbonate extractable phosphorus. Levels above 60 parts per million (ppm) are adequate for spinach growth. In the case of soils with levels below this (particularly in winter), pre-planting applications of 20-40 pounds per acre of P_2O_5 (22 to 45 kg/ha**)** are recommended in California. Baby spinach seedlings require phosphate for healthy root growth (Colomb *et al.* 2000). Phosphorus is one of the indispensable elements for plants, and the application of P fertiliser promotes root growth, enhances utilisation of soil nutrients and water by the plants, and ultimately increases crop yields (Gao et *al.* 1989 and Li *et al*. 1995). While P fertilisation is essential for improving growth and increasing crop yields, excessive P can have a negative effect on vegetable yields and quality (Jia *et al*. 1997). Although

phosphorus is indispensable for plants, plant responsiveness to P fertilisation depends on the soil available P and on the crop species.

2.4.6.3 Potassium

Soil tests can also be used to determine Potassium (K) requirements. Soil with more than 120 ppm of ammonium acetate exchangeable potassium has sufficient quantities for a crop. Approximately 63-138 kg/ha is adequate for maintaining soil fertility for fresh baby spinach in California; fertilisation rates above that level have no impact on the yield and are considered economically wasteful. Flowering, fruiting and good plant colour can be encouraged by applying potash (Britto & Kronzucker, 2008). Potassium is one of the principal plant nutrients required for crop yield and quality (William, 2007). It plays a role in physiological processes, water retention, photosynthesis, assimilation and enzyme activation. Potassium deficiency leads to a reduction in the number and size of leaves produced (Britto & Kronzucker, 2008). However, the total amount of potassium absorbed by the crop depends on the crop grown, the amount of native soil K^+, the amount of K fertiliser applied, potassium availability in the soil, environmental conditions and the crop management practices employed (Mullins & Burmester, 1998).

2.4.7 Fertiliser application methods

As a general rule, side dressing with a nitrogen fertiliser when the plants are one-third grown is recommended. Baby spinach growth is slow in the beginning and accelerates during the final 21 days before harvest (Bergquist, 2006).

2.4.8 Harvesting

Baby spinach has leaves which are relatively small (7.5-10cm) and it is harvested 35 days after planting. Harvested spinach can be kept in a polypropylene bag in a cold room (USDA, 2005).

The timing of harvesting is important as the concentrations of bioactive compounds can vary greatly through the course of the day due to variations in water content (when concentrations are given on a fresh weight basis) or in light intensity (Mozafar, 1994). Harvesting must be done with care to avoid mechanical injury to the plants which may also have an impact on the concentrations of bioactive compounds (Lucier *et al.* 2004).

2.4.9 Postharvest handling

Baby spinach is quite perishable and will yellow if stored at higher than the recommended temperatures (0º-5ºC). However, the primary cause of postharvest losses is decay associated with mechanical damage during harvest and postharvest operations. The small surface-to-weight ratio and very high respiration rate of baby spinach means that it must be cooled rapidly to prevent excessive weight loss and wilting (Ferrante *et al*. 2004). After harvest, baby spinach is sensitive to ethylene which increases yellowing and may result in leaf decay, and is also moderately sensitive to freezing injury after harvest (Allende *et al*. 2004). Strong off-odours, decay caused by breakage, and softening tissue are considered to be major problems associated with baby spinach (Medina *et al*. 2012). Experience has shown that tissue with a high respiratory rate and/or low energy reserves has a shorter shelf life (Rico *et al*. 2007).

2.4.10 Storage

The recommended storage temperature for baby spinach is 0°-5° C, and it is usually stored in polypropylene bags. In store displays, however, it is kept at higher temperatures for a maximum storage time of 10 days (Lucier *et al.* 2004).

The limited storage time of leafy vegetables is due to their relatively high respiration rates and large surface-to-volume ratio (Toledo *et al*. 2003; Wills *et al*. 1998; and Rico *et al*. 2007). However, storability can be significantly improved by lowering temperatures and increasing humidity, thereby modifying the surrounding atmosphere (Wills *et al*. 1998). The advantage of storing at a lower temperature is that it significantly slows down the loss of ascorbic acid and carotenoid content (Kalt, 2005). However, the ascorbic acid content of spinach quickly decreases during storage at ambient temperature (Beuscher *et al.* 1999), which may cause freezing injury (Wills *et al*. 1998). Too low temperature decreases metabolic rates and thereby fastens deterioration. The produce or crop will eventually wilt and change in colour or decay due to low temperature.

2.5 Summary

High intake of fruit and vegetables is known to have a positive effect on human health and helps to combat most of the degenerative diseases associated with ageing, such as heart disease, cardiovascular disease, Alzheimer's disease,

cataracts and several forms of cancer. These protective benefits have been considered to be due to the high nutritional value and high concentrations of bioactive compounds (ascorbic acid, flavonoids and carotenoids) found in vegetables, partly as a result of the antioxidative action of some of these compounds.

The presence of minerals, vitamins, pigments, phytonutrients and minerals like potassium, manganese, zinc, magnesium, iron and calcium make baby spinach a healthy crop. Various phytonutrients and pigments found in baby spinach have been shown to protect the skin from harmful rays of the sun, including Ultra-Violet rays. Growing foetuses need the folate found in baby spinach for the proper development of the nervous system. Without it, a foetus is likely to suffer from defects such as cleft palate or spina bifida.

Baby spinach needs applications of the major fertilisers (N, P and K) at the right time and in the right quantities for optimal growth and development. Harvesting must be done with care to avoid mechanical injuries that may negatively affect the concentrations of bioactive compounds. The primary cause of postharvest losses is decay associated with mechanical damage during harvest.

CHAPTER 3

Research design and methodology

3.1 Materials and methods

3.1.1 Experimental site

The trial was conducted at the Agricultural Research Council Vegetable and Ornamental Plant Institute situated about 25 km north of central Pretoria on the Moloto/KwaMhlanga Road (R573), GPS coordinates 25º 59” S; 28º 35” E. The farm covers approximately 4000 ha, of which only 650 ha is under irrigation. Baby spinach cultivar (cv.)

3.1.2 Experimental design and treatment details

Three parallel trials using N, P and K were arranged in a randomised complete block design (RCBD) with 5 treatments, each replicated 4 times. In all the experiments, the sub-plots were 2.2 x 2.2 m in size, comprising 3 rows with 10 plants each, making a total of 30 plants per sub-plot/treatment, with inter-row spacing of 20 cm, and intra-row spacing of 10 cm as shown in Plate 1.

Baby spinach cv. Ohio was planted in the seedling trays filled with planting medium on 9 March 2014. Germination took place 5 days later and transplanting was done after 2 weeks when the plants each had 4 leaves. Nitrogen and phosphorus treatments consisted of 0, 45 kg/ha, 75 kg/ha, 105 kg/ha; 120 kg/ha. Potassium treatments consisted of 0; 63 kg/ha; 85 kg/ha; 127 kg/ha; 148 kg/ha. The fertilisers were applied 2 weeks after planting. After parallel N, P and K trials, the combined NPK trial was conducted, consisting of 5 treatments, viz. 0; 30:30:48 kg/ha; 45:45:63 kg/ha; 60:60:78 kg/ha; 75:75:93 kg/ha. The rates were based on the recommended rates used in California where baby spinach is grown in abundance. All the trials were repeated twice.

The experiment was conducted on virgin soil hence no soil analysis was done. Lime ammonium nitrate (28% N kg/ha) was applied as the N fertiliser source, phosphorus was supplied in the form of super phosphate (83% P kg/ha), and potassium was supplied in the form of potassium chloride (50% K kg/ha). The baby spinach was

irrigated based on the soil moisture conditions for a period of 2.5 hours per irrigation using sprinkler irrigation.

3.1.3 Data collection

Data plants (middle row plants) were sampled at 35 days after planting (DAP). Parameters recorded were biomass production (fresh mass, dry mass, root length, fresh root mass, dry root mass), chlorophyll content, stomatal conductance above, stomatal conductance below, leaf area index (LAI), leaf protein percentage, total leaf nitrogen/phosphorus/potassium and leaf nitrate.

Plate 1: Bed of baby spinach with 5 sub-plots and 5 different treatments of N, P and K

At harvest, the fresh mass was oven dried at 35ºC for 24 hours and then weighed to determine the total biomass (dry matter). In the N, P and K treatments, the roots were washed clean of soil particles, weighed and oven dried at 35˚C for 24 hours after which the root dry mass was measured.

The chlorophyll content was measured using a non-destructive method with a Spad 502 Chlorophyll Meter designed by Minolta Camera Co. Ltd., Japan as shown in Plate 2. Chlorophyll levels are a key indicator of plant health.

Stomatal conductance was measured by means of the steady state method using the SC-1 Leaf Porometer designed by Decagon Devices USA as shown in Plate 3.

This determines stomatal conductance by measuring actual vapour flux from the leaf through the stomates.

Plate 2: Non-destructive method of measuring chlorophyll content using Spad 502 Chlorophyll Meter

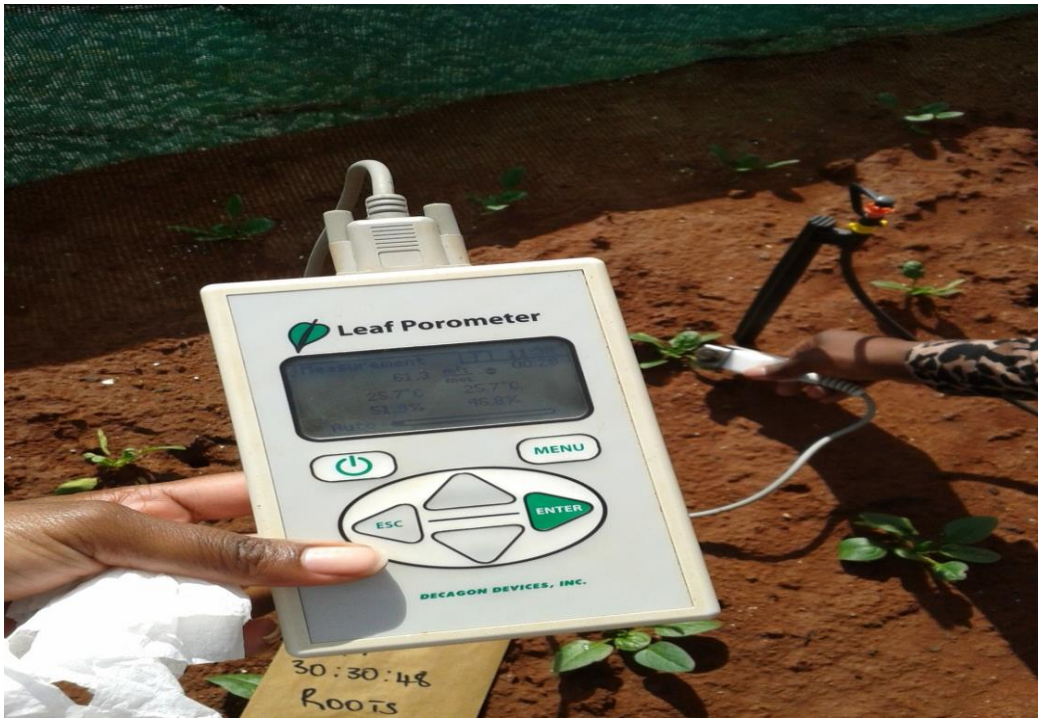

Plate 3: Leaf Porometer used to determine stomatal conductance in the leaves

Leaf area was measured using the LAI-2200C Plant Canopy Analyzer designed by Lincoln, Nebraska USA as shown in Plate 4.

Plate 4: Non-destructive LAI-2200C Plant Canopy Analyzer

3.1.3.1 Method of determining nitrogen

A Carlo Erba NA 1500 C/N/S Analyser was used directly on the samples (in finely milled or powder form). A sample of approximately 8-14 mg was weighed into a tin foil container for each determination (Igor, 1995). It is a dry oxidation method generally known as the Dumas method.

The sample and tin container were ignited at high temperature (1020ºC) in oxygen (on a chrome oxide catalyst) to produce carbon dioxide, nitrogen gas and oxides of nitrogen (plus other oxides). In this method, the gases produced pass through silvered cobalt oxide, then a column of copper (at 540ºC), which reduced the oxides of nitrogen to nitrogen gas (and removes excess free O_2). After the removal of water vapour and CO_2 by traps, the N_2 gas was finally separated from traces of any other gases by means of gas chromatography using a helium carrier gas, and was detected using a thermal conductivity detector. The instrument is calibrated against a pure organic compound of known composition. The compound chosen for our

calibration standard was the ethyl ester of 4-aminobenzoic acid, which contains 8,48% N. PeakNet software (Dionex Corporation, May 1998), with an external A/D interface (UI20 Universal Interface, Dionex) was used for data collection, peak integration, calibration and computation of concentrations (Dionex Corporation, 1998).

3.1.3.2 Leaf tissue phosphorus concentrations

Phosphorus was determined using method described by Mudau *et al*. 2005

3.1.3.3 Leaf tissue potassium concentrations

Potassium was determined using method described by Mudau *et al*. 2005

3.1.3.4 Sample extraction for nitrate and nitrite

A sub-sample of the sample was extracted with distilled water, using a 0.2 g sample to 50 ml of water and shaken on a mechanical shaker for 30 minutes before filtering. For the N control sample, only 0.096 g was used instead of 0.2 g as there was insufficient sample available. The water extract solution was analysed using ion chromatography which detects nitrate as well as most of the other major anions (nitrite, chloride, fluoride, sulphate, etc.) by separating the anions on an ion exchange column and detecting them with a conductivity detector. The nitrite was below the detection limit for all samples. The nitrate N was calculated from the N using a factor of 0.226, which is the mass of an N atom divided by the mass of NO_3 (N atom + 3 O atoms) (Zasoski & Burau, 1977).

3.1.3.5 Computation of protein N and protein

The protein N was estimated by subtracting the nitrate N from the total N. This estimate is expected to be slightly too high, because chlorophyll N, ammonium N and any other possible forms of non-protein N (other than nitrate) have not been excluded. While values for chlorophyll were available, these were on a mass/unit area basis, not a mass/mass basis; therefore without the thickness and density of the leaves, they could not be converted to a mass/unit mass basis. From the estimate of non-protein N, the protein was estimated by using a factor of 6.25. This is the default factor usually used for the conversion of N to protein when the correct factor for the product of interest is unknown. It is based on the assumption that the

protein contains 16.0% N. However, this factor of 6.25 is more appropriate for meat products. Many types of plant protein have a higher percentage of N than 16.0%, so the best protein factor for many plant products is slightly less than 6.25, and for some plant types, slightly below 6.0 (Jimenez & Ladha, 1993).

3.1.3.6 ICP-OES determination of phosphorus and potassium

An aliquot of the digest solution was used for the ICP-OES (Inductively Coupled Plasma Optical Emission Spectrometric) determination of P and K. The ICP-OES is a multi-element instrument. The instrument used (Varian Liberty Series II) is a sequential instrument, where the elements are determined almost simultaneously, with only a few seconds between each element. Each element was measured at an appropriate emission wavelength, chosen for high sensitivity and lack of spectral interference. The wavelengths used were P: 213.618 nm and K: 769.896 nm.

The instrument was set up and operated according to the procedures recommended in the instrument manual. It was calibrated against a series of standard solutions, containing all the elements of interest in the proportions found in typical leaf samples. (Unpublished method developed by Mike Philpott at ARC-ISCW based on the procedures recommended in the instrument manual (Liberty Series, 1997)).

3.1.4 Statistical analysis

Data were subjected to analysis of variance (ANOVA) using the Statistical Analysis System Version 8.0 (SAS Institute, 2003) (See Appendix 1). In all the trials, treatment sums of squares were partitioned into linear and quadratic polynomial contrasts for all the variables to be measured. The mean separation was done using Turkey's test for the sake of completeness, despite the fact that the trials were evaluating responses.

CHAPTER 4

Results and discussion

4.1 Results

4.1.1 Response of biomass production to nitrogen nutrition (Table 1)

Fresh mass

Nitrogen applied quadratically increased fresh mass, reaching maximum at 75 kg/ha. The difference between the highest and lowest mean values on fresh mass was 33.38 g per plant.

Dry mass

The dry matter showed a similar trend to that of fresh mass, reaching maximum at 75kg/ha. The difference between the highest and lowest mean values was 7.96 g per plant.

Root length

Nitrogen applied significantly increased root length (21.50 cm), reaching maximum at 75 kg/ha. The difference between the highest and lowest mean values on root length was 7.00 cm.

Fresh root mass and dry root mass

The application of different nitrogen levels did not have any significant effect on root mass.

Leaf Protein N %

Nitrogen applied linearly increased the percentage protein content to 4.26% of the baby spinach as compared to the control. The significant increases varied with the applications of 0 to 45kg/ha. The difference between the highest and lowest values was 2.90%.

Total Leaf N %

Percentage leaf nitrogen of the baby spinach showed a significant increase at 45 kg/ha. The difference between the highest value and lowest value was 3.2%.

Leaf Nitrate %

Where high nitrogen treatment was applied at 120 kg/ha, a significant percentage increase in the nitrate content level of 1.33% was observed. The difference between the highest and lowest values was 1.31%.

Leaf Protein %

All treatments linearly increased the percentage leaf protein contents compared to the control. The difference between the highest and lowest leaf protein percentage values was 18.3%.

Table 1: Biomass production parameters of baby spinach in response to nitrogen nutrition

Applied nitrogen (kg/ha)	Fresh mass (g) per plant	Dry mass (g)per plant	Root length (cm)	Fresh root mass (g)per plant	Dry root mass (g)per plant	Leaf protein N %	Total leaf N %	Leaf nitrate%	Leaf protein %*
0	12.43d	2.33c	14.50b	1.84a	0.460a	1.34b	1.34b	0.02b	8.3b
45	37.62ab	5.43b	16.00b	0.54a	0.150a	4.01a	4.01a	0.04b	25.0a
75	45.81a	10.29a	21.50a	0.47a	0.115a	4.26a	4.31a	0.23b	26.6a
105	27.89bc	10.20a	20.50a	0.92a	0.315a	4.22a	4.34a	0.57b	26.3a
120	21.49cd	8.91a	15.00b	0.37a	0.090a	4.22a	4.54a	1.33a	26.5a
LSD (5%)	13.501	3.049	3.268	2.185	0.563	2.56	2.56	0.020	15.70
Significance level	0.0014	0.0003	0.0011	0.5918	0.5804	0.001	0.012	0.023	0.001
Response	Q**	Q**	Q**	NS	NS	L**	L**	L**	L**

NS,*, **Linear or quadratic (Q) effects non-significant (ns) or significant at P ≤ 0.05 or 0.01.

4.1.2 Response of chlorophyll, stomatal conductance and Leaf Area Index to nitrogen nutrition (Table 2)

Chlorophyll content

Nitrogen treatments ranging 0 to 75 kg/ha quadratically improved chlorophyll content. The difference between the highest and lowest mean values for chlorophyll was 286.7 nm.

Stomatal conductance - adaxial (upper)

Nitrogen treatments also improved the upper stomatal conductance. Plants not fed with nitrogen did not open the stomatal conductance compared to those where nitrogen treatments were applied. The difference between the highest and lowest mean values was 382.7 mmol/m^2/s^{-1}.

Stomatal conductance - abaxial (lower)

The results showed a significant increase at very high levels of nitrogen application, reaching maximum at 120 kg/ha. The difference between the highest value and lowest mean values was 183.97 mmol/m^2/s^{-1}.

Leaf Area Index

There was a linear response to different levels of nitrogen, ranging from 0 to 120 kg/ha. The difference between the highest and lowest mean values was 1.35 per plant.

Table 2: Physiological parameters of baby spinach in response to nitrogen nutrition

Applied nitrogen (kg/ha)	Chlorophyll content (nm)	Stomatal conductance (upper) (mmol/ m^2/s)	Stomatal conductance (lower) (mmol/m^2/s)	Leaf Area Index
0	151.08c	815.0b	743.73b	2.90b
45	338.38a	907.2a	819.48a	3.58a
75	437.78a	1197.7a	781.93a	3.78a
105	370.85a	937.5a	866.30a	4.08a
120	233.50b	865.3b	921.70a	4.25a
LSD (5%)	143.73	292.46	171.9	1.1227
Significance level	0.0050	0.001	0.001	0.001
Response	Q**	Q**	L**	L**

NS,*, **Linear or quadratic (Q) effects non significant (ns) or significant at P ≤ 0.05, 0.01 or 0.001.

4.1.3 Response of biomass production to phosphorus nutrition (Table 3)

Fresh mass

The results showed that phosphorus application quadratically increased the fresh mass of baby spinach, reaching maximum at 75 kg/ha. The significant increases varied with the applications of 0 to 75 kg/ha P. The difference between the highest and lowest mean values was 10.29 g per plant.

Dry mass

The results showed that phosphorus fertiliser application quadratically increased the dry mass of baby spinach, reaching maximum of 75 kg/ha. The significant increases varied with the applications of 0 to 75 kg/ha P. The difference between the highest lowest mean values was 2.45 g per plant.

Root length

Different rates of phosphorus applied did not have a significant influence on the root length of the baby spinach.

Fresh root mass and dry root mass

Applied phosphorus did not exhibit any significant influence on either fresh or dry root mass.

Leaf Protein P %

The results showed that phosphorus applied linearly increased the leaf protein P percentage in the baby spinach leaves. Significant increases varied with the applications of 0 to 45 kg/ha. The difference between the highest and lowest leaf protein percentages was 0.42%.

Total Leaf P %

Applied phosphorus treatments linearly increased total leaf phosphorus percentage in the baby spinach. The difference between the highest total and lowest total leaf P percentages was 2.17%.

Leaf Nitrate %

Different rates of phosphorus applied did not have a significant influence on the leaf nitrate percentage of baby spinach.

Leaf Protein %

The results showed that phosphorus applied linearly increased the percentage leaf protein in the baby spinach leaves from 45 kg/ha. The difference between the highest and lowest leaf protein percentages was 13.5%.

Table 3: Biomass production parameters of baby spinach in response to phosphorus nutrition

Applied phosphorus (kg/ha)	Fresh mass (g)per plant	Dry mass (g) per plant	Root length (cm)	Fresh root mass (g) per plant	Dry root mass(g) per plant	Leaf protein P %	Total leaf P %	Leaf nitrate	Leaf protein %*
0	3.54b	0.23b	7.75a	0.21a	0.09a	0.53b	2.13b	0.02a	13.30b
45	5.69b	0.15b	5.50a	0.24a	0.11a	0.97a	3.42ab	0.06a	21.30a
75	13.04a	2.69a	10.25a	0.23a	0.11a	0.95a	4.25a	0.09a	26.40a
105	12.94a	1.21b	8.25a	0.25a	0.06a	0.95a	4.22a	0.09a	26.30a
120	13.83a	1.24b	9.75a	0.42a	0.08a	1.13a	4.32a	0.14a	26.80a
LSD (5%)	4.56	1.29	4.92	0.30	0.08	0.44	1.00	7.00	8.21
Significance level	0.0007	0.0070	0.2985	0.5865	0.6926	0.0001	0.001	0.001	0.001
Response	Q**	Q**	NS	NS	NS	L**	L**	NS	L**

NS,*, **Linear or quadratic (Q) effects non significant (ns) or significant at P ≤ 0.05 or 0.01.

4.1.4 Response of chlorophyll, stomatal conductance and Leaf Area Index to phosphorus nutrition (Table 4)

Chlorophyll content

All phosphorus treatments significantly increased the chlorophyll content of the baby spinach. The significant increases ranged from 0 to 69.00 nm. The difference between the highest and lowest mean values was 102.35 nm.

Stomatal conductance - adaxial (upper)

Adaxial stomatal conductance was not influenced by the different rates of phosphorus application.

Stomatal conductance - abaxial (lower)

Applying phosphorus at 45 kg/ha significantly influenced the abaxial stomatal conductance of the baby spinach by 71.70 mmol/m^2/s.

Leaf Area Index

Applying phosphorus at 75 kg/ha significantly improved the leaf area of the baby spinach as indicated in Table 4. The difference between the highest and lowest mean values was 13.139.

Table 4: Physiological parameters of baby spinach in response to phosphorus nutrition

Applied phosphorus (kg/ha)	Chlorophyll content (nm)	Stomatal conductance - adaxial (upper) (mmol/m^2/s)	Stomatal conductance - abaxial (lower) (mmol/m^2/s)	Leaf Area Index
0	62.78c	59.53a	65.00b	8.04b
45	69.00b	72.05a	71.70a	9.40b
75	171.35a	114.40a	64.93a	21.18a
105	90.45b	61.50a	42.23b	17.37a
120	95.28b	39.78a	53.40b	17.87a
LSD (5%)	26.66	77.67	28.244	6.1522
Significance level	<.0001	0.3575	0.2329	0.0019
Response	Q**	NS	Q**	Q**

NS,*, **Linear or quadratic (Q) effects non significant (ns) or significant at P ≤ 0.05, 0.01 or 0.001

4.1.5 Response of biomass production to potassium nutrition (Table 5)

Fresh Mass

Different rates of potassium application did not significantly influence the fresh mass of the baby spinach.

Dry mass

Different rates of potassium application did not significantly influence the dry mass of the baby spinach.

Root length

Different rates of potassium application did not significantly influence the root length of the baby spinach.

Fresh root mass and dry root mass

The application of potassium did not significantly influence fresh or dry root mass.

Leaf Protein K %

The application of potassium did not significantly influence the leaf protein K percentage.

Total Leaf K %

The application of potassium caused a linear response in the total leaf potassium percentage of the baby spinach from 63 kg/ha. The difference between the highest total leaf K % and lowest total leaf K % was 0.58.

Leaf Nitrate %

Different rates of potassium application did not significantly influence the leaf nitrate of the baby spinach.

Table 5: Biomass production parameters of baby spinach in response to potassium nutrition

Applied potassium (kg/ha)	Fresh mass (g)per plant	Dry mass (g)per plant	Root length (cm)	Fresh root mass (g)per plant	Dry root mass (g)per plant	Leaf protein K %	Total Leaf K %	Leaf nitrate %	Leaf protein %
0	1.25a	0.27a	8.000a	0.59a	0.26a	0.26a	1.74b	0.03a	10.8b
63	1.13a	0.26a	5.250a	0.37a	0.06a	0.36a	2.30a	0.08a	14.3a
85	3.69a	0.99a	9.500a	0.92a	0.16a	0.31a	2.32a	0.09a	14.4a
127	1.32a	0.24a	6.250a	0.43a	0.06a	0.15a	1.79b	0.04a	11.1b
148	1.65a	0.33a	6.000a	0.46a	0.07a	0.20a	1.83b	0.04a	11.4b
LSD (5%)	3.13	0.94	4.59	0.58	0.24	1.64	3.00	0.24	
Significance level	0.3979	0.3886	0.3118	0.2975	0.3136	0.003	0.001	0.3136	
Response	NS	NS	NS	NS	NS	NS	Q**	NS	Q**

NS,*, **Linear or quadratic (Q) effects non significant (ns) or significant at P ≤ 0.05 or 0.01.

4.1.6 Response of chlorophyll, stomatal conductance and Leaf Area Index to potassium nutrition (Table 6)

Chlorophyll content

Different rates of potassium nutrition did not significantly influence the chlorophyll content of the baby spinach.

Stomatal conductance adaxial (upper)

Different rates of potassium application did not significantly influence the stomatal conductance adaxial of the baby spinach.

Stomatal conductance abaxial (lower)

Different rates of potassium application did not significantly influence the stomatal conductance abaxial of the baby spinach.

Leaf Area Index

The application of potassium caused a quadratic response in the Leaf Area Index of the baby spinach at 63 kg/ha. The difference between the highest and lowest mean values was 0.57.

Table 6: Physiological parameters of baby spinach in response to potassium nutrition

Applied potassium (kg)	Chlorophyll content (nm)	Stomatal conductance - adaxial (upper) (mmol/m^2/s)	Stomatal conductance - abaxial (lower) (mmol/m^2/s)	Leaf Area Index
0	80.05a	32.25a	36.93a	0.91b
63	66.30a	42.00a	38.00a	1.31a
85	81.73a	39.55a	39.28a	1.02a
127	48.28a	43.03a	41.38a	1.02a
148	53.78a	48.48a	47.43a	0.74b
LSD (5%)	48.51	17.35	15.13	0.36
Significance level	0.49	0.40	0.59	0.58
Response	NS	NS	NS	Q**

NS,*, **Linear or quadratic (Q) effects non significant (ns) or significant at P ≤ 0.05, 0.01 or 0.001.

4.1.7 Response of biomass production to combined NPK nutrition (Table 7)

Fresh Mass

The combination of NPK significantly increased fresh mass compared to the control. However, combined 45:45:60 and 75:75:90 kg/ha yielded more fresh mass, reaching 8.40g. The difference between the highest and lowest mean values was 5.59 g per plant.

Dry mass

The application of NPK combination at 45:45:60 and 75:75:90 kg/ha increased the dry matter of the baby spinach, achieving 6.29 g and 5.09 g respectively. The difference between the highest and lowest mean values was 5.375 g per plant.

Root length

None of the treatments improved root length.

Fresh root mass and dry root mass

Neither fresh root mass nor dry root mass of the baby spinach were improved by the application of combined NPK.

Leaf protein percentage

The application of combined NPK significantly improved percentage leaf protein content (27.8%) at 30:30:40 kg/ha. Significant improvements varied with the 0 to 30:30:40 NPK applications. The difference between the highest and lowest leaf protein percentages was 7.8%.

Leaf percentage N

The application of NPK combination at 45:45:60 showed a quadratic response to leaf percentage N of baby spinach, achieving 4.51%. The difference between the lowest mean value and highest mean value was 1.3%.

Leaf percentage P

Different rates of NPK combined application did not exhibit a significant influence on the leaf percentage P of baby spinach.

Leaf percentage K

The application of combined NPK nutrition at 30:30:40 and 75:75:90 kg/ha showed a quadratic response in the leaf percentage K of the baby spinach, achieving 5.90% and 6.13% respectively. The difference between the highest and lowest leaf K percentages was 2.68%.

Table 7: Biomass production parameters of baby spinach in response to combined NPK nutrition

Applied NPK combined (kg/ha)	Fresh mass (g)per plant	Dry mass (g)per plant	Root length (cm)	Fresh root mass (g) per plant	Dry root mass (g) per plant	Protein %	Leaf N %	Leaf P %	Leaf K %
0	2.81c	0.91b	8.00a	0.59a	0.26a	20.0b	3.21b	0.19b	3.45b
30:30:40	6.13b	1.81b	6.50a	0.38a	0.25a	27.8a	4.51a	0.45b	5.90a
45:45:60	9.08a	6.29a	7.75a	0.50[a]	0.25[a]	25.4a	4.10a	0.49b	5.79a
60:60:70	5.91b	1.88b	7.75a	0.45[a]	0.39[a]	26.5a	4.30a	0.60b	5.86a
75:75:90	8.40ab	5.09a	8.50a	0.60a	0.21a	27.1a	4.42a	0.53b	6.13a
LSD (5%)	2.72	1.89	3.10	0.26	0.23	6.8	1.3	0.45	1.55
Significance level	0.0024	0.0001	0.7107	0.3668	0.5397	0.0001	0.003	0.012	0.023
Response	Q**	Q**	NS	NS	NS	Q**	Q**	NS	Q**

Letters in the same column are not significantly different at P≤0.0001

4.1.8 Response of chlorophyll, stomatal conductance and leaf area index to combined NPK nutrition (Table 8)

Chlorophyll content

Combined NPK applications did not exhibit a consistent trend in terms of chlorophyll content.

Stomatal conductance - adaxial (upper)

High application rates of NPK (60:60:70 kg/ha) had a significant effect on the upper stomatal conductance (38.65 mmol/m^2/s) of the baby spinach. The difference between the highest and lowest mean values was 13.38 mmol/m^2/s.

Stomatal conductance - abaxial (lower)

Excessively high application of combined NPK (75:75:90 kg/ha) significantly influenced stomatal conductance on the lower surface of the baby spinach leaves, reaching 42.85 mmol/m^2/s. The difference between the highest and lowest mean values was 12.4 mmol/m^2/s.

Leaf Area Index

No significant differences in leaf area were observed following applications of combined NPK nutrition.

Table 8: Physiological parameters of baby spinach in response to combined NPK nutrition

Applied NPK combined (kg/ha)	Chlorophyll content (nm)	Stomatal conductance - adaxial (upper) (mmol/m^2/s)	Stomatal conductance - abaxial (lower) (mmol/m^2/s)	Leaf Area Index
0	179.85ab	34.35b	35.68b	1.09a
30:30:40	156.30b	26.63b	34.18b	0.84a
45:45:60	176.60ab	25.28b	33.65b	1.11a
60:60:70	188.25a	38.65a	30.45b	0.95a
75:75:90	172.95ab	28.80b	42.85a	0.77a
Significance Level	0.1974	0.0907	0.2551	0.6518
Response	NS	Q**	Q**	NS

Letters in the same column are not significantly different at P≤0.0001

4.2 Discussion

4.2.1 Response of yield and dry matter to nitrogen nutrition

The results of this study showed that fresh mass of baby spinach was negatively affected by excessively high or excessively low nitrogen treatments. Nitrogen application levels that were too high, at 120 kg/ha, or zero nitrogen application resulted in a reduction in growth rate leading to smaller leaves. These results were consistent with the findings of Soundy *et al*. (2001) who reported that nitrogen deficiency reduced lettuce yields. Soundy & Cantliffe (2001) also reported that increasing N concentrations resulted in increased shoot growth in lettuce plantlets. Wang *et al*. (2000) reported that increased N fertiliser application by farmers increased their lettuce yields by 200 kg/ha. Moreover, the results of this study suggest that baby spinach cv. Ohio reached maximum yield at nitrogen levels of 75 kg/ha. These results were consistent with those of Collins & McCoy (1997) and Stagnari *et al*. (2007), who reported that crop production and growth were promoted by adequate supply of N. Luo *et al*. (2008) and Cooke *et al*. (2003) also found that N fertilisation of native spinach at a rate of 200 kg/ha resulted in higher photosynthetic rates, higher leaf chlorophyll concentrations and larger leaves. In contrast with our findings, Hammad *et al*. (2007) reported an increase in fresh mass of spinach aerial organs following high nitrogen application rates.

In the present experiment, the dry matter of baby spinach was significantly reduced when nitrogen was not applied (the control) and when the nitrogen application rate was very high (120 kg/ha), due to nitrogen deficiency and osmotic stress respectively. Cantliffe (1992) and Magnifico *et al*. (1992) reported that spinach is extremely responsive to nitrogen fertilisation which leads to fast vegetative growth. These findings were similar to those of Elia *et al*. (1999), who suggested that a decrease in dry matter content was caused by increased application of nitrogen fertiliser. Santamaria *et al*. (1999) also stated that the reduction in dry matter content could be attributed to the replacement of organic acids and sugars by nitrate caused by increased nitrogen fertiliser applications. On the other hand, Schuphan (1969) reported that the dry matter in spinach decreased with increasing amounts of nitrogen fertiliser.

The root length of the baby spinach was significantly affected when an adequate rate of nitrogen (at 75 kg/ha) was applied. These results were consistent with the

findings of Kamh *et al*. (2005) who reported that adequate application of nitrogen led to an absolute increase in the roots of spinach of more than 50%. But, Novaes *et al*. (2009) also found that high biomass production above the ground, induced by high N nutrition, inhibited root growth and led to a decrease in the length of the root (shoot root). Reidenbach & Horst (1997) indicated that the efficiency of nitrogen uptake depended on root morphology and uptake ability in *Brassica* crops. Wiesler & Horst (1994) and Oikeh *et al*. (1999) reported that better uptake of N from the soil was easier in cultivars with a deeper root system and higher root density.

The results of this study showed that high nitrogen applications (at 120 kg/ha) had a positive effect on the nitrate content found in the leaves of the baby spinach (1.33 mg/kg). These results were consistent with those of Briemer (1982); Elia *et al*. (1999); Gulser (2005) and Hammad *et al.* (2007) who reported that high nitrogen applications increased nitrate accumulation in vegetables. Pavlovic *et al*. (1996) and Gastal & Lemaine (2002) found that spinach has a high concentration of nitrate. Maynard *et* al. (1976) and Tei *et al*. (2000) also reported that baby spinach tends to accumulate more NO_3^- than other vegetables because of its efficient uptake system versus an inefficient reductive system. The level of nitrate in the leaves is significant due to the fact that too high nitrate is detrimental to human if consumed.

However, Stagnari *et al*. (2007) reported the detrimental impact of nitrate found in baby spinach on human health; it was found to induce methemoglobinemia which is very harmful to babies. However, Gulser (2005) reported that nitrate content in the leaves was determined by the cultivar or variety of spinach rather than the amount of nitrogen applied. In Germany, a maximum of 791 mg nitrate-Nkg^{-1} is given as acceptable nitrate content for fresh spinach (Hammad *et al*. (2007). The European Commission (1997) issued a regulation stating that the maximum acceptable level of nitrate accumulation in spinach was between 2500 and 3000 ppm on fresh weight basis (Santamaria *et al*. 1998). The World Health Organisation (WHO, 2003) recommends a level of 2500 mg/kg of nitrate in spinach, cabbage and lettuce. Cil and Katkat, (1995) proposed 700 mg nitrate-Nkg^{-1} as the maximum for leafy and root vegetables. The current study met the acceptable nitrate standard by achieving 1.33 mg/kg which is below the recommended rate.

4.2.2 Response of chlorophyll, stomatal conductance and Leaf Area Index to nitrogen nutrition

The chlorophyll content of the baby spinach increased with increasing nitrogen applications, reaching maximum at 75 kg/ha, while it significantly decreased at application rates of 105-120 kg/ha. These findings were consistent with the results reported by Van Lerser, (1999); James & Van Lerser (2001) and Kang & Van Lerser (2002) who observed that a decrease in chlorophyll content resulted from low and very high concentrations of N nutrition, due to lower N levels in the leaves. Yellowing or loss of green colour, which is considered the major consequence of chlorophyll degradation, was also observed with nitrogen levels that were too high and with zero nitrogen. Nitrogen applications above 75 kg/ha lead to osmotic stress and consequently to a decrease in N uptake, which may also affect the biosynthesis of plant metabolites, including chlorophyll. These findings from the current research indicated that adequate application of nitrogen fertilizers increased chlorophyll content of baby spinach contradict those of Hortensteiner, (2006); Majunmadar *et al.* (1991) and Ni *et al*. (2001) who reported that biotic and abiotic stresses such as water stress, heat stress, insect feeding and ageing caused chlorophyll degradation.

Adaxial (upper) stomatal conductance in the baby spinach was not significantly influenced by adequate application of nitrogen fertiliser level, only showing a slight increase at 75 kg/ha. In this study, abaxial (lower) stomatal conductance started showing an increase at the highest rate of nitrogen application (120 kg/ha). There was no difference in the total stomatal conductance on both adaxial and abaxial leaf surfaces between the different nitrogen treatments. The results of this study concur with those of Cantin *et al*. (1997) and Samuelson (2000) who reported that increased efficiency in water use by leaves, through decreased stomatal conductance and increased net photosynthesis, was caused by nitrogen nutrition. Whitehead & Hoxton (1952) also stated that more complex factors than the plant's response to fertilisation (such as temperature) may influence stomatal opening and closing.

The results of the current study showed that the application of nitrogen did not have any effect on the leaf area of baby spinach. Although increasing the nitrogen application rates to 105 and 120 kg/ha slightly increased the leaf area, the change was not significant. These results contradict those of Taylor *et al*. (1993) and Gastal

& Lemaire (2002) who reported that N application has an effect on cell division and cell expansion which can lead to an increase in leaf area. Nitrogen nutrition strongly affects the growth rate of young leaves, and has an effect on leaf area which can lead to cell expansion and cell division. A major consequence of N deficiency in plants is a decreased growth rate.

4.2.3 Response of fresh mass and dry mass to phosphorus nutrition

In this study, adequate phosphorus (at 75 kg/ha) and excessive phosphorus (at 120 kg/ha) proved to have a significant influence on the yield of baby spinach. These results are in contrast to those of Maschner (1995) who stated that stunted plant growth and cell and leaf reduction resulted from phosphorus deficiency. Jia *et al*. (1997) stated that the yield and growth of vegetables were improved and increased by supplying P, while too much P fertiliser could have a negative effect on vegetable yield and quality. Azcon *et al*. (1996) supported the above finding that increased growth, glutamine synthesis activity and protein content of lettuce was achieved by the application of P at adequate rate of 16.4% and high rate of 25.1%.

Dry matter seemed to be significantly affected by an adequate supply of phosphorus, reaching maximum at 75 kg/ha. These results were similar to those of Khokar & Warsi (1987) who reported that the application of phosphorus resulted in an increase in dry matter accumulation of up to 60% (Plenets *et al*. 2000).

4.2.4 Response of chlorophyll, stomatal conductance and Leaf Area Index to phosphorus nutrition

An adequate supply of phosphorus at 75 kg/ha led to a significant increase in the chlorophyll content of the baby spinach, while insufficient or excessive phosphorus application resulted in reduced chlorophyll content. These results were similar to those reported by Xu *et al*. (2007) who found a 40% reduction in chlorophyll and protein content as a result of insufficient application of phosphorus. Sharma (1995) also observed a higher rate of photosynthesis and chlorophyll content when phosphorus was applied as compared to the control. These results agree with Sinha *et al*. (1995) reported that P applications increased chlorophyll levels by 21% due to an increase in the synthesis of chlorophyll a and b under adequate moisture conditions (Nyborg *et al*. 1999).

The different rates of phosphorus did not have any significant influence on the leaf area of the baby spinach, which consequently led to a low crop yield. This study also showed that zero phosphorus reduced the leaf area of baby spinach, and subsequently also the yield.

These results were consistent with those of Fredeen *et al*. (1989) who stated that reduction in leaf expansion, number of leaves and leaf surface area are common symptoms of phosphorus deficiency which often result in limited yield. Reymond *et a*l. (2006) also reported a significant effect of phosphorus on leaf area, although preceded by an increase in root hydraulic conductance which resulted in an increase in soil water extraction and consequently the maintenance of greater leaf water potential under conditions of soil moisture deficit.

According to the results of this study, none of the different phosphorus application rates had a significant influence on the upper stomatal conductance of the baby spinach. These results differed from those reported by Longnecker (1994) who found an increase in cell expansion of crops as a result of phosphorus application. Garg *et al*. (2001) also reported that water deficits caused genotype variation in net photosynthetic rate, and this was attributed to the relationship between carbon dioxide concentration inside the leaf and the stomatal aperture.

The results of this study also showed no significant effect on the root length of baby spinach by any of the phosphorus application rates. However, this was contrary to reports by Borch *et al*. (1999); Miller *et al*. (2003) and Lynch *et al*. (2006) that adventitious roots in many crops are regulated by over 70% by the availability of phosphorus. Zhu *et al*. (2005a) reported that lateral roots play an important role in phosphorus acquisition by increasing soil exploration, the absorptive surface of the root system and phosphorus solubilisation. However, it should be noted that the current study focused only on root length, without differentiating between lateral or adventitious roots.

4.2.5 Response of fresh mass and dry mass to potassium nutrition

Our results showed that the application of K did not significantly increase or improve biomass production. These results were consistent with the findings of Soundy *et al.* (2001) who reported that application of K^+ to lettuce did not have a significant effect on fresh mass, dry mass, leaf area or other growth parameters. Tiwari *et al*. (1998)

also reported that the availability of K^+ to plants is usually limited, which leads to severely restricted plant growth and yield, although K^+ is considered to be one of the most abundant soil elements. Contrary to these results, Singh and Blanke, (2000) reported that an increase in the chlorophyll content, root length, yield and dry matter in *Brassica oleracea* crops resulted from an increase in K^+ fertiliser. William (2007) reported that a reduction in both the number of leaves produced and the size of individual leaves were the common symptoms of potassium deficiency.

The potassium fertiliser applied did not have any significant influence on the opening and closing of stomata. These results are consistent with those of Pervez *et al*. (2004) and Benlloch-Gonzalez *et al*. (2010) who reported that, under well-watered conditions, potassium application had no effect on stomatal conductance whereas K^+ starvation could favour stomatal opening and promote transpiration under drought stress. Jin *et al*. (2011) and Tomemori *et al*. (2002) also reported stomatal closure and inhibited photosynthetic rates in several crops as a result of K^+ deficiency. Benlloch-Gonzalez *et al*. (2010) found that plants with low K^+ status could inhibit water stress which may consequently induce stomatal closure through ethylene synthesis. Inhibitor (cobalt) and stomatal conductance could be significantly reduced in K^+ starved plants which could in turn inhibit the action of abscicic acid (ABA) on stomata and delay stomata closure. ABA is an endogenous anti-transpirant that reduces water loss through stomatal pores on the leaf surface (Tomemori *et al*., 2002).

On the other hand, Maschner (1995); Schachtman & Shin (2007); and Wang & Wu (2013) reported that plant growth and development is greatly influenced by high rate of potassium applications. Conversely, Maschner (2012) also found that turgor regulation within the guard cells during stomatal movement resulted from potassium sufficiency in the plant. Bednarz *et a*l. (1998) reported that at the onset of a developing potassium deficiency, stomatal conductance was the principal factor limiting photosynthesis, whereas when the K^+ deficiency became more extreme, non-stomatal or biochemical factors became the overriding reason for the decrease in photosynthesis. Yadav *et al*. (1999) also reported that potassium played a significant role in the opening and closing of stomatal cionductance under adequate light conditions, the guard cells produced abundant ATP in photosynthetic phosphorylation, thus supporting the active potassium uptake with sufficient energy which resulted in high turgor pressure and caused the opening of the stomata.

Talbott & Zeiger (1996) observed that stomatal opening in the course of the day was a two-phase process, with K^+ promoting opening early in the day and then giving way to sucrose as the principal driving osmotic force around midday. Berdnarz *et al*. (1998); Huber, (1985) and Longstreth & Nobel (1980) observed a relationship between stomatal aperture and plants with K^+ guard cell concentration. Insufficient levels of K^+ in the leaf can lead to decreased stomatal conductance, which also leads to decreased photosynthesis per unit leaf area (Berdnarz *et al*. 1998).

4.2.6 Response of chlorophyll, stomatal conductance and Leaf Area Index to potassium nutrition

An increase in K^+ was observed to have a significant effect on the Leaf Area Index which reached maximum at 63 kg/ha of potassium. Similarly, Huber (1985) reported that the symptoms of insufficient K^+ levels often resulted in reduced leaf area expansion, leading to reduced leaf size. Decreased leaf area often results in an increase in the concentration of cellular components and nutrients on the leaf area compared to leaves with adequate K^+ levels. Zhao *et al*. (2001); Degl'Innocenti *et al*. (2009); and Gerardeaux *et al*. (2010) also reported that reduction in leaf area is caused by K^+ deficiency. Jordan-Meille & Pellerin (2004) found that the decrease in leaf area caused by a K^+ deficiency often resulted in a reduction in biomass.

The results of the study also showed that when K^+ was not applied (the control) and when K^+ applications were too high, there was a decline in leaf area, resulting in small size leaves. These results concur with those reported by Cassman, (1998); Ebelhar & Varsa (2000); Heckman & Kamprath (1995); Mullins *et al*. (1994); and Pettigrew & Meredith, (1997) who found that reduction in plant stature was the first visual and obvious symptom of insufficient levels of potassium in the plant. Conversely however, Gwathmey & Howard (1998); and Pettigrew (2003) reported that a reduction in canopy and sunlight interception were the factors that caused leaf area reduction and consequently a reduction in the number of leaves.

4.2.7 Response of fresh mass and dry mass to NPK combined nutrition

There is little literature on the effects of combined NPK on baby spinach. This study attempts to elucidate the interactive effects of NPK on biomass production and the physiological response of baby spinach.

The results of the study showed that adequate applications of NPK (at 45:45:60 kg/ha) had a significant effect on the yield of baby spinach. Increasing the rates of combined NPK did not have any further influence on the yield. These results were consistent with the findings of various authors such as Collins & McCoy (1997); and Stagnari *et al*. (2007), who reported that the yield of many crops was significantly increased when nitrogen was applied at an adequate rate of 60 kg/ha. Jia *et al*. (1997) and Azcon *et al*. (1996) stated that the application of phosphorus had a positive effect on yield, and Singh & Blanke (2000) found an increase in chlorophyll content, root length, yield and dry matter in *Brassica oleracea* crops as a result of increased applications of K^+ fertiliser. Elia *et al*. (1999) found a decrease in dry matter content as a result of an increase in nitrogen fertiliser, and Khokar & Warsi (1987) reported that phosphorus fertiliser resulted in an increase in the dry matter accumulation.

CHAPTER 5

CONCLUSION AND RECOMMENDATIONS

The growth and development of baby spinach is dependent on the adequate application of nitrogen (N), phosphorus (P) and potassium (K) fertilisers, correct spacing and irrigation. Correct harvesting techniques and careful postharvest handling are also important in preventing damage that may negatively affect production and yield.

Different cultivars of baby spinach perform differently in terms of yield; the current study focused on the Ohio cultivar.

It can be concluded that the growth and development of baby spinach were significantly affected by the fertilisers applied as well as fertiliser rates. The results also suggest that adequate application of nitrogen and phosphorus fertiliser at 75 kg/ha improves plant growth parameters significantly, leading to a quadratic effect on yield, dry matter, chlorophyll content and leaf area. It was also observed that different rates of potassium application had a negative effect on the yield, dry matter, stomatal conductance and chlorophyll content of baby spinach, but not on leaf area. Combined NPK fertilisers applied at a rate of 45:45:60 kg/ha had a significant effect on the yield as well as dry matter of baby spinach. No application and very high rates had negative effects on baby spinach growth.

Based on the findings of this study, the recommended fertilisers for optimum yield, dry matter, chlorophyll, leaf area and stomatal conductance in baby spinach are nitrogen and phosphorus applied at a rate of 75 kg/ha. At this stage, no recommendations can be made in terms of potassium. In the case of combined NPK fertilisers, a rate of 45:45:60 kg/ha is recommended. The price of the combined fertilizers should be affordable to the farmers because of the size of the bag, the smaller the bag, the smaller the price

Further studies to determine the economic impact of these rates should be conducted.

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APPENDIX 1

Analysis of variance (ANOVA) for parameters of N, P, K and NPK combined nutrition

ANOVA for fresh mass in response to nitrogen

Source	DF	Sum of Squares	Mean Square	F Value	Pr>F
Model	7	3167.791510	452.541644	5.89	0.0038
Error	12	921.568570	76.797381		
TRT	4	2756.267230	689.066808	8.97	0.0014
BLOCK	3	411.524280	137.174760	1.79	0.2033
Corrected Total	19	4089.360080			

ANOVA for dry mass in response to nitrogen

Source	DF	Sum of Squares	Mean Square	F Value	Pr>F
Model	7	207.6550700	29.6650100	7.57	0.0013
Error	12	47.0043300	3.9170275		
TRT	4	192.1973500	48.0493375	12.27	0.0003
BLOCK	3	15.4577200	5.1525733	1.32	0.3149
Corrected Total	19	254.6594000			

ANOVA for root length in response to nitrogen

Source	DF	Sum of Squares	Mean Square	F Value	Pr>F
Model	7	505.0000000	72.1428571	16.03	<.0001
Error	12	54.0000000	4.5000000		
TRT	4	170.0000000	42.5000000	9.44	0.0011
BLOCK	3	335.0000000	111.6666667	24.81	<.0001
Corrected Total	19	559.0000000			

ANOVA for fresh root mass in response to nitrogen

Source	DF	Sum of Squares	Mean Square	F Value	Pr>F
Model	7	11.89420000	1.69917143	0.84	0.5723
Error	12	24.13548000	2.01129000		
TRT	4	5.82868000	1.45717000	0.72	0.5918
BLOCK	3	6.06552000	2.02184000	1.01	0.4241
Corrected Total	19	36.02968000			

ANOVA for dry root mass in response to nitrogen

Source	DF	Sum of Squares	Mean Square	F Value	Pr>F
Model	7	0.94628000	0.13518286	1.01	0.4689
Error	12	1.60180000	0.13348333		
TRT	4	0.39708000	0.09927000	0.74	0.5804
BLOCK	3	0.54920000	0.18306667	1.37	0.2986
Corrected Total	19	2.54808000			

ANOVA for chlorophyll in response to nitrogen

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	7	254251.7430	36321.6776	3.89	0.0192
Error	12	112106.1890	9324.1824		
TRT	4	243952.3870	60988.0968	6.53	0.0050
BLOCK	3	10299.3560	3433.1187	0.37	0.7778
Corrected Total	19	366357.9320			

ANOVA for upper stomatal conductance in response to nitrogen

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	7	380591.5575	54370.2225	1.51	0.2534
Error	12	432411.6000	36034.3000		
TRT	4	354383.9400	88595.9850	2.46	0.1020
BLOCK	3	26207.6175	8735.8725	0.24	0.8651
Corrected Total	19	813003.1575			

ANOVA for lower stomatal conductance in response to nitrogen

Source	DF	Sum of Squares	Mean Square	F Value	Pr>F
Model	7	127362.0925	18194.5846	1.46	0.2688
Error	12	149398.8850	12449.9071		
TRT	4	78138.93500	19534.98375	1.57	0.2452
BLOCK	3	49222.15750	16407.38583	1.32	0.3141
Corrected Total	19	276760.9775			

ANOVA for Leaf Area Index (LAI) in response to nitrogen

Source	DF	Sum of Squares	Mean Square	F Value	Pr>F
Model	7	5.60094135	0.80013448	1.51	0.2540
Error	12	6.37198920	0.53099910		
TRT	4	4.41228680	2.08	2.08	0.1470
BLOCK	3	1.18865455	0.75	0.75	0.5451
Corrected Total	19	11.97293055			

ANOVA for fresh mass in response to phosphorus

Source	DF	Sum of Squares	Mean Square	F Value	Pr>F
Model	7	382.6431300	57.6633043	6.25	0.0030
Error	12	104.9649500	8.7470792		
TRT	4	370.9469300	92.7367325	10.60	0.0007
BLOCK	3	11.6962000	3.8987333	0.45	0.7248
Corrected Total	19	487.6080800			

ANOVA for dry matter in response to phosphorus

Source	DF	Sum of Squares	Mean Square	F Value	Pr>F
Model	7	18.97504000	2.71072000	3.85	0.0199
Error	12	8.44244000	0.70353667		
TRT	4	16.81968000	4.20492000	5.98	0.0070
BLOCK	3	2.15536000	0.71845333	1.02	0.4176
Corrected Total	19	27.41748000			

ANOVA for root length in response to phosphorus

Source	DF	Sum of Squares	Mean Square	F Value	Pr>F
Model	7	138.0000000	19.7142857	1.94	0.1503
Error	12	122.2000000	10.1833333		
TRT	4	56.20000000	14.05000000	1.38	0.2985
BLOCK	3	81.80000000	27.26666667	2.68	0.0943
Corrected Total	19	260.2000000			

ANOVA for fresh root mass in response to phosphorus

Source	DF	Sum of Squares	Mean Square	F Value	Pr>F
Model	7	1.14912500	0.16416071	4.35	0.0127
Error	12	0.45253000	0.03771083		
TRT	4	0.11063000	0.02765750	0.73	0.5865
BLOCK	3	1.03849500	0.34616500	9.18	0.0020
Corrected Total	19	1.60165500			

ANOVA for dry root mass in response to phosphorus

Source	DF	Sum of Squares	Mean Square	F Value	Pr>F
Model	7	0.09225000	0.01317857	4.35	0.0128
Error	12	0.03635000	0.00302917		
TRT	4	0.00685000	0.00171250	0.57	0.6926
BLOCK	3	0.08540000	0.02846667	9.40	0.0018
Corrected Total	19	0.12860000			

ANOVA for chlorophyll in response to phosphorus

Source	DF	Sum of Squares	Mean Square	F Value	Pr>F
Model	7	36451.34100	5207.33443	17.39	<.0001
Error	12	3592.36100	299.36342		
TRT	4	30104.74700	7526.18675	25.14	<.0001
BLOCK	3	6346.59400	2115.53133	7.07	0.0054
Corrected Total	19	40043.70200			

ANOVA for upper stomatal conductance in response to phosphorus

Source	DF	Sum of Squares	Mean Square	F Value	Pr>F
Model	7	14657.86300	2093.98043	0.82	0.5863
Error	12	30498.24700	2541.52058		
TRT	4	12278.30500	3069.57625	1.21	0.3575
BLOCK	3	2379.55800	793.18600	0.31	0.8163
Corrected Total	19	45156.11000			

ANOVA for lower stomatal conductance in response to phosphorus

Source	DF	Sum of Squares	Mean Square	F Value	Pr>F
Model	7	5588.721000	798.388714	2.38	0.0900
Error	12	4032.929000	336.077417		
TRT	4	2176.575000	544.143750	1.62	0.2329
BLOCK	3	3412.146000	1137.382000	3.38	0.0541
Corrected Total	19	9621.650000			

ANOVA for Leaf Area Index in response to phosphorus

Source	DF	Sum of Squares	Mean Square	F Value	Pr>F
Model	7	574.8939522	82.1277074	5.15	0.0066
Error	12	191.3527944	15.9460662		
TRT	4	526.3385088	131.5846272	8.25	0.0019
BLOCK	3	48.5554434	16.1851478	1.01	0.4201
Corrected Total	19	766.2467466			

ANOVA for fresh mass in response to potassium

Source	DF	Sum of Squares	Mean Square	F Value	Pr>F
Model	7	26.05603000	3.72229000	0.90	0.5365
Error	12	49.61925000	4.13493750		
TRT	4	18.29703000	4.57425750	1.11	0.3979
BLOCK	3	7.75900000	2.58633333	0.63	0.6122
Corrected Total	19	7.75900000			

ANOVA for dry mass in response to potassium

Source	DF	Sum of Squares	Mean Square	F Value	Pr>F
Model	7	2.73720500	0.39102929	0.05	0.4488
Error	12	4.47489000	0.37290750		
TRT	4	1.68347000	0.42086750	1.13	0.3886
BLOCK	3	1.05373500	0.35124500	0.94	0.4509
Corrected Total	19	7.21209500			

ANOVA for root length in response to potassium

Source	DF	Sum of Squares	Mean Square	F Value	Pr>F
Model	7	53.5000000	7.6428571	0.86	0.5616
Error	12	106.5000000	8.8750000		
TRT	4	47.50000000	11.87500000	1.34	0.3118
BLOCK	3	6.00000000	2.00000000	0.23	0.8769
Corrected Total	19	160.0000000			

ANOVA for fresh root mass in response to potassium

Source	DF	Sum of Squares	Mean Square	F Value	Pr>F
Model	7	0.87177660	0.12453951	0.89	0.5438
Error	12	1.68152160	0.14012680		
TRT	4	0.77511520	0.19377880	1.38	0.2975
BLOCK	3	0.09666140	0.3222047	0.23	0.8738
Corrected Total	19	2.55329820			

ANOVA for dry roots mass in response to potassium

Source	DF	Sum of Squares	Mean Square	F Value	Pr>F
Model	7	0.20034500	0.02862071	1.19	0.3790
Error	12	0.28963000	0.02413583		
TRT	4	0.12865000	0.03216250	1.33	0.3136
BLOCK	3	0.07169500	0.02389833	0.99	0.4303
Corrected Total	19	0.48997500			

ANOVA for chlorophyll in response to potassium

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	7	4169.19050	595.59864	0.60	0.7448
Error	12	11896.32700	991.36058		
TRT	4	3633.565000	908.391250	0.92	0.4857
BLOCK	3	535.625500	178.541833	0.18	0.9078
Corrected Total	19	16065.51750			

ANOVA for upper stomatal conductance in response to potassium

Source	DF	Sum of Squares	Mean Square	F Value	Pr> F
Model	7	1170.873000	167.267571	1.32	0.3212
Error	12	1522.315000	126.859583		
TRT	4	558.4930000	139.6232500	1.10	0.4003
BLOCK	3	612.3800000	204.1266667	1.61	0.2391
Corrected Total	19	2693.188000			

ANOVA for lower stomatal conductance in response to potassium

Source	DF	Sum of Squares	Mean Square	F Value	Pr>F
Model	7	589.626000	84.232286	0.87	0.5538
Error	12	1157.674000	96.472833		
TRT	4	276.8100000	69.2025000	0.72	0.5962
BLOCK	3	312.8160000	104.2720000	1.08	0.3942
Corrected Total	19	1747.300000			

ANOVA for Leaf Area Index (LAI) in response to potassium

Source	DF	Sum of Squares	Mean Square	F Value	Pr>F
Model	7	1.62758410	0.23251201	4.25	0.0139
Error	12	0.65655810	0.05471317		
TRT	4	0.67715670	0.16928918	3.09	0.0576
BLOCK	3	0.95042740	0.31680913	5.79	0.0110
Corrected Total	19	2.28414220			

ANOVA for fresh mass in response to NPK interaction

Source	DF	Sum of Squares	Mean Square	F Value	Pr>F
Model	7	128.5904200	18.3700600	5.90	0.0038
Error	12	37.3830000	3.1152500		
TRT	4	97.55452000	24.38863000	7.83	0.0024
BLOCK	3	31.03590000	10.34530000	3.32	0.0568
Corrected Total	19	165.9734200			

ANOVA for dry mass in response to NPK interaction

Source	DF	Sum of Squares	Mean Square	F Value	Pr>F
Model	7	95.5979200	13.6568457	9.21	0.0005
Error	12	17.7967000	1.4830583		
TRT	4	88.12182000	22.03045500	14.85	0.0001
BLOCK	3	7.47610000	2.49203333	1.68	0.2239
Corrected Total	19	113.3946200			

ANOVA for root length in response to NPK interaction

Source	DF	Sum of Squares	Mean Square	F Value	Pr>F
Model	7	15.70000000	2.24285714	0.55	0.7782
Error	12	48.50000000	4.04166667		
TRT	4	8.70000000	2.17500000	0.54	0.7107
BLOCK	3	7.00000000	2.33333333	0.58	0.6409
Corrected Total	19	64.20000000			

ANOVA for fresh root mass in response to NPK interaction

Source	DF	Sum of Squares	Mean Square	F Value	Pr>F
Model	7	0.20165000	0.02880714	0.99	0.4794
Error	12	0.34765000	0.02897083		
TRT	4	0.13715000	0.03428750	1.18	0.3668
BLOCK	3	0.06450000	0.02150000	0.74	0.5472
Corrected Total	19	0.54930000			

ANOVA for dry root mass in response to NPK interaction

Source	DF	Sum of Squares	Mean Square	F Value	Pr>F
Model	7	0.20004500	0.02857786	1.32	0.3221
Error	12	0.26053000	0.02171083		
TRT	4	0.07075000	0.01768750	0.81	0.5397
BLOCK	3	0.12929500	0.04309833	1.99	0.1701
Corrected Total	19	0.46057500			

ANOVA for chlorophyll in response to NPK interaction

Source	DF	Sum of Squares	Mean Square	F Value	Pr>F
Model	7	3413.869050	487.695579	1.56	0.2365
Error	12	3741.269530	311.772461		
TRT	4	2221.267430	555.316857	1.78	0.1974
BLOCK	3	1192.601620	397.533873	1.28	0.3272
Corrected Total	19	7155.138580			

ANOVA for upper stomatal conductance in response to NPK interaction

Source	DF	Sum of Squares	Mean Square	F Value	Pr>F
Model	7	1243.069000	177.581286	3.64	0.0243
Error	12	585.699000	48.808250		
TRT	4	504.6530000	126.1632500	2.58	0.0907
BLOCK	3	738.4160000	246.1386667	5.04	0.0173
Corrected Total	19	1828.768000			

ANOVA for lower stomatal conductance in response to NPK interaction

Source	DF	Sum of Squares	Mean Square	F Value	Pr>F
Model	7	678.371000	96.910143	1.75	0.1875
Error	12	663.457000	55.288083		
TRT	4	338.5430000	84.6357500	1.53	0.2551
BLOCK	3	339.8280000	113.2760000	2.05	0.1607
Corrected Total	19	1341.828000			

ANOVA for Leaf Area Index in response to NPK interaction

Source	DF	Sum of Squares	Mean Square	F Value	Pr>F
Model	7	1.88252500	0.26893214	1.87	0.1630
Error	12	1.72756320	0.14396360		
TRT	4	0.36157920	0.09039480	0.63	0.6518
BLOCK	3	1.52094580	0.50698193	3.52	0.0489
Corrected Total	19	3.361008820			