# YIELD AND QUALITY RESPONSES OF EGYPTIAN WHITE GARLIC (*ALLIUM SATIVUM* L.) AND WILD GARLIC (*TULBAGHIA VIOLACEA* HARV.*)* TO NITROGEN NUTRITION

**NYENGEDZENI MUDZIWA**



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# Yield and quality responses of Egyptian white garlic (*Allium sativum* L.) and wild garlic (*Tulbaghia violacea* Harv.) to nitrogen nutrition

**by**

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## DECLARATION

I, the undersigned, hereby declare that the dissertation submitted herewith for the degree (M Inst Agrar: Horticultural Science) to the University of Pretoria contains my own independent work and has not been submitted for any degree at any other University. I further declare that all sources cited are indicated and acknowledged by means of a comprehensive list of references.

| ---------------------------------- | --------------------------------------- |
|---|---|
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**Yields and quality responses of Egyptian white garlic (*Allium sativum* L.) and wild garlic (*Tulbaghia violacea* Harv.) to nitrogen nutrition**

By

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Supervisor: Prof P. Soundy

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**Abstract**

*Allium sativum* and *Tulbaghia violacea* are some of the most important medicinal plants used by South African traditional healers for the treatment of flu, fever, cold, tuberculosis, asthma and many more diseases. However, growth, yield and quality are constrained by excessive and under fertilization. This study was carried out to determine, firstly, the effect of N source (ammonium sulphate and calcium nitrate) on yield and quality of *A. sativum* and *T. violacea* plants. Secondly, to determine the best season for harvesting *T. violacea* and lastly, to determine the antifungal effects of *A. sativum* and *T. violacea* plant extracts against plant pathogens *Altenaria solani* and *Sclerotium rolfsii*. Both plants were treated with both N sources applied as topdressing treatments at a total of 0, 50, 100, 150 and 200 $kg{\cdot}ha^{-1}$, divided into three applications at three week (*A. sativum*) and three month (*T. violacea*) intervals. *A. sativum* plants were sampled at 54, 82, 112, 140 and 175 days after planting (DAP) while, *T. violacea* plants were sampled monthly for ten months. Parameters recorded were growth analysis, yield and bioactivity for both plant species. Both nitrogen sources improved plant growth and yield of *A. sativum* and *T. violacea* plants. Calcium nitrate at 150 $kg{\cdot}ha^{-1}$ and ammonium sulphate at 200 $kg{\cdot}ha^{-1}$ produced the highest at 24 $t{\cdot}ha^{-1}$ and 27 $t{\cdot}ha^{-1}$, respectively. Ammonium sulphate improved bioactivity of leaves with the highest bioactivity recorded at 82 and 112 DAP.

Yield obtained from the autumn harvest was not affected by N source. Ammonium sulphate and calcium nitrate at 200 $kg{\cdot}ha^{-1}$ produced the highest yields of 23.6 $t{\cdot}ha^{-1}$ and 23.5 $t{\cdot}ha^{-1}$, respectively. In contrast, yield obtained

from the winter harvest was affected by N source at 200 $kg \cdot ha^{-1}$, with significantly better yield of 30.8 $t \cdot ha^{-1}$ with calcium nitrate compared to 27.4 $t \cdot ha^{-1}$ with ammonium sulphate. Crude extracts of *T. violacea* bulbs that were treated with ammonium sulphate significantly inhibited the growth of plant pathogenic fungi, whereas extracts from plants treated with calcium nitrate showed low bioactivity. Extracts from plants grown with ammonium sulphate at 100 $kg \cdot ha^{-1}$ were more effective in controlling growth of plant pathogens when compared to other N levels. The minimum inhibitory concentration (MIC) effects of *A. sativum* against *S. rolfsii* and *A. solani* were at 0.01 $mg \cdot mL^{-1}$. The MIC of *T. violacea* extracts against *A. solani* was at 0.006 $mg \cdot mL^{-1}$. The MIC of *T. violacea* extracts were better than previously reported in literature. Therefore, *A. sativum* and *T. violacea* plant extracts can be used as fungicides against *S. rolfsii* and *A. solani* diseases for crops such as tomato and potato.

**Keywords**: *Allium sativum, Alternaria solani*, ammonium sulphate, calcium nitrate, *Sclerotium rolfsii*, *Tulbaghia violacea*

## GENERAL INTRODUCTION

Egyptian white garlic (*Allium sativum*) is a bulbous annual plant and wild garlic (*Tulbaghia violacea*) is a bulbous perennial plant. Both these plants belong to the Alliaceae family (Van Wyk, Van Oudtshoorn & Gericke, 1997). Both of these plants share a common character of producing a strong smell when crushed due to their natural properties (Lindsey & Van Staden, 2004).

*A. sativum* originated in Central Asia and spread to Southwest Asia and the Mediterranean region. It has been domesticated and used for human consumption over 10 million years ago (Kamenetsky & Rabinowitch, 2006). *A. sativum* is one of the most important vegetable crops grown throughout the world (Lanzotti, 2006). It is second in the rank after onion because of its important nutritional value in human diet and its cultivation (Tabor, Getahun & Zelleke, 2004). The best season to grow the *A. sativum* plant is during winter when it is cool (Stork, Potgieter, Van den Heever, & Niederwieser, 2004). It is well adapted for the production in all parts of South Africa especially in cool and dry regions; areas include Polokwane planteau and Northern Cape (Douglas area). It requires rich soil, good drainage and 500 mm of water for its growing period (Coertze & Stork, 1998). Water should not be deficient during bulb formation until two weeks before harvesting time. Excess supply of water at two weeks before harvesting affects the storage quality (Potgieter, 2006). The crop prefers a soil with a pH between 6.5 and 7.5, but a soil pH lower than 6.0 and higher than 8.0 can affect yield (Boyhan, Kelly & Granberry, 2000). Kamenetsky & Rabinowitch (2006) reported that *A. sativum* plants are normally propagated vegetatively from bulbs by means of separation of cloves. Its growth period is between 160 to 180 days (Stork *et al.*, 2004).

Van Wyk *et al.* (1997) reported that *T. violacea* originated in Kwa-Zulu Natal, Free State and Eastern Cape provinces. *T. violacea* has been used by South African communities as a herb for many years (Harris, 2004). It is one of the ten most important medicinal plants that are used by traditional health practitioners (Van Wyk *et al.,* 1997) and were found growing by their own in

the wild. It grows in a wide range of soil types including rocky soils (Harris, 2004) and it can be grown throughout the year. It takes more than two growing seasons of growth to complete its cycle (Stork *et al*., 2004). *T. violacea* is propagated either by its seeds or by division of the large clumps (Van den Heever, 2006). Bodnar, Schumacher & Uyenaka (2004) reported that *A. sativum* could be used in food, tomato sauce, soups, stews, salads and breads and it is a major part in the daily diet of many families throughout the world. Zulus use *T. violacea* leaves as spinach. The bulbs are not eaten but mainly used for medicinal purposes (Harris, 2004). Van Wyk *et al*. (1997) reported that excessive consumption and utilization of *T. violacea* plants causes abdominal pains. Both of these plants have medicinal properties because of their antifungal, antibacterial and antiviral activities (Rode, De Wet & Cywes, 1989; Lindsey & Van Staden, 2004; Nteso & Pretorius, 2006).

Davidson (2002) reported that consumption of *A. sativum* contribute to the prevention of many diseases. Both plants were found to be the most efficient natural treatments of hypertension, arteriosclerosis, heart dysfunctions, cholesterol, diabetes, cancer, gout, arthritis and obesity (Pamplona-Roger & Malaxetxebarria, 2004). These plant species were successful when they were used as a complement in the treatment of HIV/AIDS (Tshabalala, 2006). They have been used as fungicides to control fungal infection on vegetables crops (Nteso & Pretorius, 2006).

The demand of *T. violacea* is increasing very fast and this will lead to local extinction of the plants. Currently, there is a problem of over-exploitation of many important medicinal plants in some of the provinces or regions of South Africa (Van Wyk *et al*., 1997). According to the South African Garlic Growers Association (SAGGA) of South Africa, fresh produce garlic market is around 2 600 tones per year (Stork *et al*., 2004). The demand of *A. sativum* bulbs is increasing in South Africa due to the awareness of its medicinal values and its economic importance (Adekpe, Shebayan & Miko, 2007). High amount of *A. sativum* bulbs is sold in Gauteng and Kwa-Zulu Natal, Eastern Cape and Limpopo. The price on the local market varies from R5.00 to R30.00 per kg. The best price is obtained when *A. sativum* bulb is in short supply (Stork *et*

*al*., 2004). A 2 g serving of *A. sativum* will provide potassium, carbohydrate, and trace amounts of calcium, fiber, iron and vitamin C (Dickerson, 1999), as well as fat, protein, thiamine, riboflavin, nicotinamide and ascorbic acid (Tindall, 1967). *T. violacea* has been cultivated as a source of fungicides and as a potential plant for horticultural purposes due to its attractive flowers (Stork *et al*., 2004).

In order to improve *A. sativum* production the following factors should be considered: planting time, fertilizer application (time and rate) and optimal plant population (Brewster & Butler, 1989). The production of vigorous sprouts is one of the most important factors of successful *A. sativum* production (Potgieter, 2006). Adequate application of nitrogen during sprouting stage promotes vigorous vegetative growth and optimum leaf expansion (Stork *et al*., 2004). Excessive application of nitrogen at a late vegetative stage of *A. sativum* crop can limit yields and increases storage losses (Brewster & Butler, 1989), while inadequate N can hasten maturity and limit yield (Batal, Bondari, Granberry & Mulinix, 1994).

The application of different sources of nitrogen plays an important role in production of vigorous vegetative plants. Nitrogen sources and levels influence bulb size produced. It is important for farmers to classify the needs of the targeted market in order to meet them. Normally, the demand from the market is for small, medium and large bulbs size while extra small bulbs are produced for processing (Stork *et al*., 2004).

The aim of this study was to:

a) determine the effect of N source (ammonium sulphate and calcium nitrate) on yield and quality of *A. sativum* and *T. violacea*,
b) determine the best season for harvesting *T. violacea* and
c) determine the antifungal effects of *A. sativum* and *T. violacea* plant extracts against plant pathogens *Altenaria solani* and *Sclerotium rolfsii*.

# CHAPTER 1

# LITERATURE REVIEW

## 1.1 Importance of medicinal plants

Medicinal plants (locally called *mushonga* in Tshivenda language, *Uumuti* in Zulu language and *moreane* in Sotho language) are an important part of the health care system in Africa since the olden times (Wiersum, Dold, Husselman & Cocks, 2006). There is a heavy dependence on medicinal plants in Africa because of their relative accessibility, low prices, local availability and acceptability by local communities (Jagtap, Deokule & Bhosle, 2006). In addition, many Africans are residing in rural areas and are located far from hospitals or clinics and transport facilities are not often available.

The production of medicinal plants offers a possibility of upgrading the lives and well being of people in rural areas (Mander, 1997). The use of medicinal plants is not found only in rural areas or used by people who earn low income, but is extending even in urban areas (Wiersum *et al.*, 2006).

There are informal and formal markets selling medicinal plants and these have been established throughout the local communities and are contributing towards the multimillion rands generated annually in South Africa (Wiersum *et al.*, 2006). Trading in traditional medicines is stimulated by high population growth rates, rapid urbanization and realisation of their cultural value. An estimated amount of 20 000 tonnes of plant materials are harvested from the wild (Dold & Cocks, 2002) and are valued at approximately R270 million annually (Coetzee, Jefthas & Reinten, 1999; Wiersum *et al.*, 2006). The figures above are a conservative indication of what is being traded in every city, town, village and informal settlement across all provinces of South Africa (Dold & Cocks, 2002). For example at Witwatersrand, Gauteng Province, there are about fifty shops selling *umuthi* that were surveyed (Williams, Blakwill & Witkowski, 2000). About 511 plant species that were identified in the fifty *umuthi* shops at Witwatersrand were based on the race-groups of the

herb-traders. The number of plant species increases daily due to the fact that each day there is new plant species entering the market (Williams *et al.*, 2000). Most race-groups were Indians, Whites and Africans, and received a regular income from the plant species, which are known to be on demand and frequently purchased by their consumers (Williams *et al.*, 2000).

### 1.2 Conservation measures for medicinal plants

Demand for medicinal plants has lead to increasing pressure on wild plant populations. Many plant species in South Africa are now facing local extinction. In South Africa and worldwide, sustainable harvest of medicinal plants from wild populations is the most misunderstood and misused concept in today's field conservation (Giday, Asfaw, Elmqvist & Woldu, 2003).

Cultivation of targeted plant species is the most preferable option that has been initiated by South Africa for conservation and sustainable use of medicinal plants (Mosunkutu, 2007). Significant efforts have been made to develop community gardens and nurseries for cultivation of medicinal plants (Cunningham, 2001). Therefore, cultivation of medicinal plants is considered as an alternative way than to collect plant materials from the wild.

Certain medicinal plant species have been cultivated at Nqabara in the Eastern Cape (Table 1.1) in order to avoid plant extinction. Some of the plant species were found suitable (S) for cultivation but others were unsuitable (U) (Wiersum *et al.*, 2006). Plant species that were unsuitable for cultivation was as a result of some ecological reasons. Some of the tree species grew fast and became huge trees, which consumed a lot of water and space (Kelrungi & Fabriclus, 2005). The application of high amounts of fertilizer and water apparently dilutes the healing power of a plant. Some plants are difficult to multiply or propagate, either by stem cuttings or seed germination (Wiersum *et al.*, 2006). Plant species that were in danger of extinction at Nqabara were *Protorhus longifolia, Rhoicissus digitata, Rhoicissus tridentate* and *Llex mitis* (Dold & Cocks, 2002; Kelrungi & Fabriclus, 2005). The above-mentioned plants are currently scarce, due to heavy trading and there is a problem of

unsustainable harvest because they are traded at a high price in South Africa (Coetzee *et al.*, 1999; Dold & Cocks, 2002; Wiersum *et al.*, 2006).

Table 1.1 The most important 17 medicinal plants in the Eastern Cape area, their form, parts used and local opinions about their suitability (S) and unsuitable (U) for cultivation (Kelrungi & Fabriclus, 2005)

| Plant species | Plant form | Parts used by traditional healers | Suitable (S) and unsuitable (U) for cultivation |
|---|---|---|---|
| *Strychnos henningsii* | Tree | Bark | U |
| *Araujia sericifera* | Climber | Roots | S |
| *Behnia reticulata* | Climber | Tuber | S |
| *Protorhus longifolia* | Tree | Bark | U |
| *Stangeria eriopus* | Woody suffrutex | Root | S |
| *Schotia latifolia* | Tree | Bark | U |
| *Rhoicissus digitata* | Climber | Tuber | S |
| *Artemisia afra* | Herb | Leaves | S |
| *Pittoporum viridiflorum* | Tree | Stem bark | S |
| *Llex mitis* | Tree | Bark and leaves | U |
| *Aloe ferox* | Tree | Leaves and rind | S |
| *Rhoicissus tridentata* | Climber | Tuber | S |
| *Potamogeton thunbergii* | Aquatic herb | Leaves | S |
| *Acalypha glabrata* | Tree | Bark | U |
| *Schotia afra* | Tree | Bark | S |
| *Zanthoxylum davyi* | Tree | Bark | U |
| *Agapanthus africanus* | Geophyte | Roots | U |

Traditional healers prefer different plant parts, such as roots, bark, tubers and leaves. Certain plant parts of medicinal plants collected can create a serious threat to the survival of the species (Zschocke, Rabe, Taylor, Jager & Van Staden, 2000). Substitution with a plant part of the same species, on the other

hand, is likely to be much better accepted by the patients of traditional healers (Van Wyk *et al*., 1997). It is not possible to enforce laws forbidding gatherers from harvesting the bark and underground parts of the protected plants, but a change in the requirements of the healers will be a reason for the gatherers to primarily collect leaves, twigs or other aerial parts of the plant (Zschocke *et al*., 2000).

Poto (2007) suggested a close interaction with traditional healers and the cultivators of medicinal plants, so that they must protect more plant species from extinction, and allow the recovery of threatened medicinal plants**.** The barks of many different tree species have been intensively and frequently harvested from the wild (Diederichs, Geldenhuys & Mitchell, 2002). Ring barking of trees makes a tree to die back and the plant species become rare over a period of time (Figure 1.1). The figure below is indicating over harvesting of the bark. The lower barks of this plant have been extensively removed. Currently, collectors are using stepladders to harvest the higher or upper barks. The lower part of this tree is drying out and it is indicating that in all areas where the bark has been harvested it is going to dry out. Thereafter, the whole tree will die.

Figure 1.1 Illustration of intensive and frequent harvesting of bark from a tree species (Diederichs *et al.,* 2002).

Conservation of medicinal plants can assist in protecting the environment, habitats and biodiversity in South Africa (Poto, 2007). Implementation of projects that will focus on medicinal plant species, which are commonly used in South Africa by traditional healers, is important. Therefore, a better coordination and cooperation should be practised among various agencies such as Herbalists, Healers, Foresters and Pharmaceuticals regarding utilization and regeneration of selected medicinal plants that are required to overcome the problem of plant extinctions (Mosunkutu, 2007). To conserve biodiversity and avoid overexploitation, there should be a prerequisite for safeguarding access to traditional medicine and encouraging its further development (Timmermans, 2003). Sustainable harvesting of medicinal plants can be done effectively through training of local collectors (traditional doctors, diviners, traditional birth attendants and traditional surgeons) and owners of medicinal plant nurseries to harvest sustainably from the wild and nurseries (Shinwari & Gilani, 2003).

### 1.3 The new conservation Act

Department of Water Affairs and Forestry (DWAF) issued the General Licence under sections 7, 15 & 23 of National Forests Act [No. 84 of 1998] to the Associations, for harvesting of bulb or tuber, stem and bark under guidance of the management plan for natural forests in the country. The management plan provides guidelines for resource harvesting, planting for alternative resources, and monitoring of resource use impacts, and stipulates the arrangements between DWAF and the Associations. Interestingly, harvesters from other countries they have to join the Associations, and an allied Association has been established in South Africa in order to have better management plan for harvesting of medicinal plants.

### 1.4 Botanical description of *A. sativum* and *T. violacea*

#### 1.4.1 Nomenclature

Lanzotti, (2006) reported that *Allium* is one of the largest and most important representative genus of the Alliaceae family. It comprises of 450 species, and

most of them are perennial plants with underground storage bulbs. Most of them have high economic significance, including vegetables such as *A. sativum* (garlic), *A. cepa* (bulb onion and shallot), *A. ampeloprasum* (leek), *A. schoenoprasum* (chives) (Kamenetsky & Rabinowitch, 2006).

*Tulbaghia* is a small genus of about twenty species from South Africa (Jacobsen, Yamaguchi, Mann & Howard, 1967). The genus is very closely related morphologiclly to *Allium* genus, and it is a member of the Alliaceae family. The genus includes species such as *T. capensis, T. cominsii, T. dregeana, T. galpinii, T. simmleri* and *T. acutiloba* which is found on dry rocky grassland in the Eastern Cape region (Nguyen, 2008). The common name of *T. violacea* is wild garlic, society garlic or sweet garlic (Kubec, Velisek & Musah, 2002) and in Afrikaans it is called wildeknoflok and wilde knoffel (Harris, 2004).

### 1.4.2 Distribution

*Allium sativum* originated in Central Asia, its cultivation has spread to Southwest Asia and the Mediterranean region. It has been used since the olden times on the $15^{th}$ – $17^{th}$ century (Kamenetsky & Rabinowitch, 2006). *Tulbagia violacea* Harv. originated in South Africa. It is commonly found in Natal, Gauteng and the Eastern Cape regions of South Africa (Van Wyk *et al*., 1997; Kubec *et al*., *2002*; Harris, 2004).

### 1.4.3 Morphology

*A. sativum* is an annual bulbous plant and therefore, it grows vegetatively for one growing season and at the end of the growing season the plant dies back. It is a complex bulb because it has numerous bulblets, called cloves. Cloves are formed only on the basal plate and fleshy, thick specialized scales. When the bulb is matured or at the end of the growing season, leaf sheath dry out and form the enveloping dry skins, which do not contain storage materials (Kamenetsky & Rabinowitch, 2006). The topsets (flower stalks) develop from the centre of the bulb and are grouped together into a globular head (Stork *et*

*al*., 2004). When the bulb is matured, plants usually enter a dormancy period that lasts for a few weeks to a number of months, followed by sprouting either in autumn or spring (Stork *et al*., 2004). *A. sativum* is propagated vegetatively from bulblets and topsets (Kamenetsky & Rabinowitch, 2006).

*T. violacea* is a perennial plant, and this plant can live for more than two years. It has evergreen leaves, which smell like garlic when crushed and have a culture of being used as a substitute for garlic and chives (Kubec *et al*., 2002). The plant is a fast-growing, bulbous plant that can reach a height of 0.5 m (Harris, 2004). It bears tubular flowers that are pinkish mauve in colour, and are clustered into umbels of up to twenty flowers that are above the leaves on a tall flower stalk and appear from January to April (Harris, 2004). The fruits are triangular capsules that are grouped into a head, and when ripe they split to release the flattened, hard black seeds. *T. violacea* is propagated either by seed or by division method of the large clumps of bulbs which can be planted individually (Van den Heever, 2006).

### 1.5 Traditional and local uses of *A. sativum* and *T. violacea*

In South Africa and other developing countries, mothers are the real care givers to their family by treating diseases with the use of medicinal plants (Gedif & Hahn, 2003). *T. violacea* extracts is used to treat fever and colds, asthma, tuberculosis and intestinal gastroenteritis ailments (Kubec *et al*., 2002). Leaves have been used to treat cancer of the oesophagus (Van Wyk *et al*., 1997). Crushed leaves may be used to cure sinus, headaches and to prevent moles entering gardens due to the strong smell (Kulkarni, Sparg & Van Staden, 2005).

*T. violacea* extracts are used not only for healing physical illnesses, but also have important cultural values. Some cultures in South Africa use the extracts from this plant for both healing and for protection against misfortunes with natural and supernatural causes (Hutchings, Scott, Lewis & Cunningham, 1996). The smell repels fleas, ticks and mosquitoes when crushed on the skin (Harris, 2004). *T. violavea* is a very good snake repellent and for this reason

South African people plant it around their homes as a protector (Hutchings *et al*., 1996).

Zulus use the leaves and flowers of *T. violacea* as a vegetable and as a peppery spice with meat and potatoes. Both leaves and flowers can be used in salads and in other dishes (Harris, 2004). The extensive consumption of this plant has been associated with a variety of undesirable symptoms, such as abdominal pain, inflammation of the intestine and gastroenteritis (Van Wyk *et al*., 1997).

*T. violacea* plant can be used as an ornamental plant and utilized as a nursery flower. It has high potential of been grown due to beautiful flowers with large heads of lavender blooms on tall stems that flower from mid-summer to early autumn (Stork *et al*., 2004).

*A. sativum* plant extracts (both bulb and leaves) have been used to treat bacterial, fungal and viral infections in the past years (Kaye, Nossaman, Ibrahim, Feng, Mcnamara, Agrawal & Kadowitz, 1995) and is also being used as an immune system booster. In recent years, it has successfully being used as a complement in the treatment of HIV/AIDS (Pamplona-Roger & Malaxetxebarria, 2004; Tshabalala, 2006).

*A. sativum* is a good treatment for hypertension, arteriosclerosis, heart dysfunctions, cholesterol, diabetes, cancer, gout, arthritis, certain rheumatic afflictions and obesity. In general *A. sativum* and *T. violacea* extracts are an all-purpose plant stimulants for the body, supplying health and a sense of well-being (Pamplona-Roger & Malaxetxebarria, 2004).

### 1.6. Active compounds in *A. sativum* and *T. violacea*

*A. sativum* and *T. violacea* plant extracts serve as natural sources for several active compounds (Borris, 1996). Natural compounds accumulated in plant extracts serve as growth regulators or have clear ecological role such as

protection against fungal, viral and bacterial diseases (Pretorious, Magama & Zietsman, 2003).

Several investigations indicate that the biological and medicinal activities of *A. sativum* and *A. cepa* are mainly due to their high organo-sulphur compounds content. The sulphur-containing constituents in *Allium* crops are the *S*-alk(en)yl-L-cysteine suphoxides (Corzo-Martinez, Corzo & Villamiel, 2007) (Figure 1.2). One of these organo-sulphur compounds that accumulates in *A. sativum* and *T. violacea* plant extracts has been identified as allicin (Baustista, Movahed, Hinman, Axelsson, Sterner, Hogestatt, Jullius, Jordt & Zygmunt, 2005). Garlic extracts are the breakdown products of allicin, including diallyl trisulphide (DATS) and ajoene, which have a greater antifungal effect than allicin (Corzo-Martinez *et al*., 2007). Allicin is generated by a chemical reaction catalysed by the vacuolar enzyme, alliinase (Baustista *et al*., 2005). Allicin is active against microbial infections caused by fungus, virus and bacteria both in human and plant pathogens. Allicin restrain the growth of microbial organisms due to its chemical reaction with the thiol groups of various enzymes (Baustista *et al*., 2005).

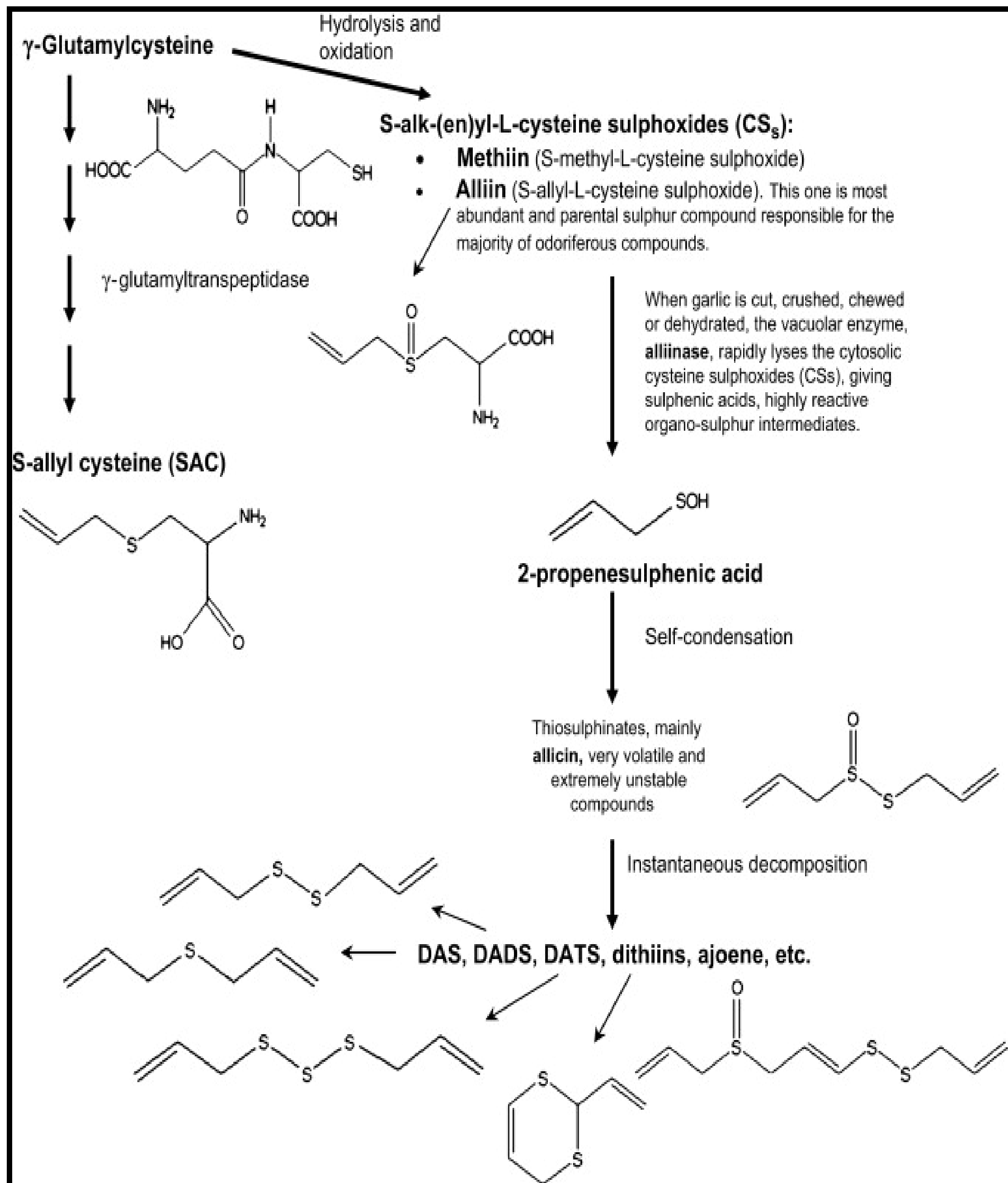


Figure 1.2 Formation of organo-sulphur compounds during metabolic pathways in processed garlic (Corzo-Martinez *et al* ., 2007)

# CHAPTER 2

# YIELD AND QUALITY RESPONSE OF EGYPTIAN WHITE GARLIC (*ALLIUM SATIVUM* L.) TO NITROGEN NUTRITION

## 2.1 INTRODUCTION

The use of fertilizers to obtain high yield and good quality are important features in today's *Allium sativum* (garlic) production. Both of these features can be improved through nitrogen (N) and sulphur (S) application strategies as influenced by the source of N and S, as well as rates and times (Luo, Branlard, Griffin & Mcneil, 2000). An increase in S supply is related to an increase in alliin content of leaves and bulbs of *A. sativum* crop (Van den Heever, 2006), whereas nitrogen fertilization has only a minor influence on crop quality (Bloem, Haneklaus & Schnug, 2004).

Kakara, Abdullahzai, Saleem & Shah (2002) reported that nitrogen accounts for a higher percentage of variation in plant height, leaf area, leaf count, and fresh and dry plant mass when it was increased from 50 to 200 $kg \cdot ha^{-1}$. Therefore, use of nitrogen fertilizer is necessary for ensuring successful vegetative growth of *A. sativum.*

Brewster & Butler (1989) reported that high levels of N nutrition prevented or delayed flowering in *Allium cepa*. Late applications of N may delay bulb ripening and give rise to thick-necked crops. However, N applied early in crop growth may accelerate maturity in irrigated crops. Stork *et al.* (2004) reported that the application of N with additional S at an early vegetative (sprouting) stage is useful for the promotion of strong vegetative growth before cold winter months.
The study was conducted to determine the ideal ammonium sulphate and calcium nitrate levels for optimal plant growth and yield, and to determine N and S accumulation from different treatments at different stages of the crop's development.

## 2.2 MATERIALS AND METHODS

### 2.2.1 Experimental site

The field experiment was conducted at the Experimental Farm of the Agricultural Research Council - Vegetable and Ornamental Plant Institute (ARC-VOPI) at Roodeplaat, Pretoria. The experiment was conducted from April to October 2006. A completely randomized block design consisting of nine treatments and four replications were used.

Soil samples were collected before planting for soil pH analysis, extractable cations and extractable phosphorus using 1:1 water, ammonium acetate and Bray-1 methods, respectively. The soil type was Oakleaf with the following composition: 0.05% nitrogen, 1330 $mg{\cdot}kg^{-1}$ calcium, 456 $mg{\cdot}kg^{-1}$ magnesium, 178 $mg{\cdot}kg^{-1}$ potassium, 117.74 $mg{\cdot}kg^{-1}$ sulphate, 78.9 $mg{\cdot}kg^{-1}$phosphorus and a soil pH of 7.5.

### 2.2.2 Plant material

*A. sativum* planting materials were obtained from Agribez Company based in Polokwane, Limpopo Province. Bulbs of the same weight and circumference were obtained. Each bulb were separated into cloves, which were classified into sizes ranging from 0–2, 2–3, 3–4.5, 4.5–6.5 and above 6.5 g. Different sized cloves have presumably an influence on yield and quality. Therefore, only cloves of 4.5–6.5 g were used as planting materials for the whole trial. Planting of *A. sativum* trial was done on the 11$^{th}$ April 2006. The gross plot size was 4.2 $m^2$, with a spacing of 10 cm within rows and 30 cm between the rows. There were 140 plants per plot, and the plant population was 333 333 plants per hectare.

### 2.2.3 Experimental design and treatment details

Application of nitrogen to *A. sativum* was commenced from the early stages of growth in order to influence the vegetative stage. It was applied by means of

top dressing at 3 week intervals starting from two weeks after emergence (Table 2.1).

Table 2.1 Source and amount of nitrogen applied to *A. sativum* at three week intervals

| N-source | Nitrogen ($kg \cdot ha^{-1}$) | 2 weeks after emergence ($g \cdot plot^{-1}$) | 5 weeks after emergence ($g \cdot plot^{-1}$) | 8 weeks after emergence ($g \cdot plot^{-1}$) | Total amount applied ($g \cdot plot^{-1}$) |
|---|---|---|---|---|---|
| Control | 0 | 0 | 0 | 0 | 0 |
| $(NH4)_2SO_4$ | 50 | 6.3 | 7.4 | 7.4 | 21.0 |
| $(NH4)_2SO_4$ | 100 | 10.4 | 15.8 | 15.8 | 42.0 |
| $(NH4)_2SO_4$ | 150 | 15.8 | 23.6 | 23.6 | 63.0 |
| $(NH4)_2SO_4$ | 200 | 21.0 | 31.5 | 31.5 | 84.0 |
| $Ca(NO_3)_2$ | 50 | 7.6 | 11.3 | 11.3 | 30.2 |
| $Ca(NO_3)_2$ | 100 | 15.1 | 22.7 | 22.7 | 60.4 |
| $Ca(NO_3)_2$ | 150 | 22.7 | 34.0 | 34.0 | 90.7 |
| $Ca(NO_3)_2$ | 200 | 30.3 | 45.3 | 45.3 | 120.9 |

Growth analysis of *A. sativum* was conducted by counting the number of leaves, measuring plant height (cm), fresh and dry leaf mass (g), fresh and dry bulb mass (g), neck and bulb circumference (mm) and leaf area ($cm^2$).

Six plants randomly selected per plot were destructively harvested at 54, 82, 112, 140 and 175 days after planting (DAP) during the winter season. At the end of the growing season, plants were harvested during spring. A total of 2376 plants were harvested for yield and quality determination of the bulbs.

**2.2.4 Statistical analysis**

Harvested plants were stored in an air forced oven for two weeks at 40 $^oC$ to dry-out. The dried samples were divided into two halves, for chemical analysis and screening of bioactivity. Collected data was sent to the ARC - Institute of Agricultural Engineering (Biometry Unit) for statistical analysis using GenStat

(2005). Differences between treatments were tested by means of analysis of variance (ANOVA). Treatment means were sum of square partinied it to linear and quadratical polynomial contrast.

## 2.3 RESULTS AND DISCUSSION

The quality of *A. sativum* and its derived products is related to the amounts of allicin or bioactivity and its biogenetic precursors, which will be discussed in chapter 3.

### 2.3.1 Gross analysis

Plant height, leaf area/plant, fresh and dry leaf mass, neck circumference, bulb yield, fresh and dry bulb mass, leaf and bulb tissue nitrogen, leaf and bulb tissue sulphur significantly increased with the increase of nitrogen fertilizer up to 200 $kg{\cdot}ha^{-1}$ ammonium sulphate and calcium nitrate.

Plant height increased significantly with an increasing ammonium sulphate and calcium nitrate application regardless of the sampling date. Plants which were harvested 112, 140 and 175 DAP showed the highest increase in plant height than those harvested at 54 and 82 DAP (Tables 2.2 and 2.3). At the final harvest (175 DAP) plants that received 50 to 200 $kg{\cdot}ha^{-1}$ ammonium sulphate or calcium nitrate 101.1 to 105, 106.3 to 112.3, respectively and had grown much taller than the control plants. The maximum plant height 105 mm at 200 ($kg{\cdot}ha^{-1}$) and 112.3 mm (200 $kg{\cdot}ha^{-1}$) applied. The different between the lowest and the highest N applied was 27 mm calcium nitrate and 20 ammonium sulphate.

Leaf area increased in a linear fashion with increasing ammonium sulphate and calcium nitrate application for the whole growing period (Tables 2.2 and 2.3). The highest values of leaf area were obtained from 150 and 200 $kg{\cdot}ha^{-1}$ ammonium sulphate 527 to 459 and calcium nitrate 540 to 612, regardless of the sampling date. The maximum leaf area 527 $cm^2$ (150 $kg{\cdot}ha^{-1}$) ammonium sulphate and 612.6 $cm^2$ (200 $kg{\cdot}ha^{-1}$) calcium nitrate. The different between

the lowest and the highest was 220 $cm^2$ ammonium sulphate and 306.1 $cm^2$ calcium nitrate.

Neck circumference is an important parameter that determines the availability of macro nutrients in the soil (Potgieter, 2006) and the production of *A. sativum*. Average neck circumference increased from 25.4 to 60.8 mm for plants produced with ammonium sulphate and 25.4 to 60.9 mm for plants produced with calcium nitrate and harvested from 54 to 175 DAP, respectively (Tables 2.2 and 2.3). The maximum neck circumference of 60.8 (200 $kg \cdot ha^{-1}$) ammonium sulphate and the maximum neck circumference 60.9 (200 $kg \cdot ha^{-1}$) calcium nitrate.

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Table 2.2 Effect of ammonium sulphate fertilizer on plant height, leaf area, neck circumference, leaf and bulb nitrogen (N) and sulphur (S)

| N levels ($kg{\cdot}ha^{-1}$) | Plant height (mm) | Leaf area ($cm^2$) | Neck circumference (mm) | Leaf tissue N $g{\cdot}kg^{-1}$ | Bulb tissue N $g{\cdot}kg^{-1}$ | Leaf tissue S $g{\cdot}kg^{-1}$ | Bulb tissue S $g{\cdot}kg^{-1}$ |
|---|---|---|---|---|---|---|---|
| | | | *54 days of planting* | | | | |
| 0 | 41.1 | 56.9 | 25.4 | 1.58 | 0.23 | 0.70 | 0.17 |
| 50 | 42.7 | 65.9 | 27.9 | 1.66 | 0.47 | 0.80 | 0.18 |
| 100 | 43.7 | 74.3 | 27.4 | 1.70 | 0.60 | 0.86 | 0.18 |
| 150 | 43.9 | 74.5 | 30.9 | 1.71 | 0.64 | 0.86 | 0.19 |
| 200 | 46.6 | 76.4 | 32.0 | 1.96 | 0.77 | 0.91 | 0.21 |
| *Response* | L** | L** | L** | L** | L** | L** | L** |
| | | | *82 days after planting* | | | | |
| 0 | 45.9 | 102 | 31.2 | 1.14 | 0.43 | 0.68 | 0.25 |
| 50 | 46.2 | 129 | 35.2 | 1.49 | 0.63 | 0.87 | 0.26 |
| 100 | 50.9 | 157 | 38.3 | 2.12 | 0.68 | 0.90 | 0.29 |
| 150 | 51.5 | 158 | 38.5 | 2.32 | 0.74 | 0.92 | 0.30 |
| 200 | 53.2 | 179 | 41.3 | 2.41 | 0.86 | 0.94 | 0.30 |
| *Response* | L** | L** | L** | L** | L** | L** | L** |
| | | | *112 days after planting* | | | | |
| 0 | 63.9 | 245 | 44.9 | 1.89 | 0.43 | 0.54 | 0.28 |
| 50 | 69.3 | 284 | 51.8 | 2.06 | 0.94 | 0.69 | 0.32 |
| 100 | 71.3 | 349 | 52.7 | 2.21 | 0.96 | 0.72 | 0.34 |
| 150 | 71.8 | 364 | 53.7 | 2.28 | 0.98 | 0.82 | 0.37 |
| 200 | 76.1 | 356 | 53.5 | 2.45 | 1.24 | 0.82 | 0.38 |
| *Response* | L** | L** | Q* | L** | L** | L* | L* |
| | | | *140 days after planting* | | | | |
| 0 | 85.7 | 313 | 52.1 | 2.24 | 0.58 | 0.52 | 0.32 |
| 50 | 86.9 | 354 | 53.0 | 2.36 | 0.96 | 0.53 | 0.33 |
| 100 | 94.7 | 368 | 54.3 | 2.76 | 0.99 | 0.58 | 0.34 |
| 150 | 97.2 | 435 | 58.9 | 2.90 | 1.18 | 0.62 | 0.39 |
| 200 | 98.3 | 472 | 60.8 | 3.03 | 1.35 | 0.64 | 0.47 |
| *Response* | L** | L** | L** | L** | L** | L** | L** |
| | | | *175 days after planting* | | | | |
| 0 | 85.6 | 307 | 30.8 | 0.41 | 1.27 | 0.25 | 0.54 |
| 50 | 101.1 | 413 | 35.5 | 0.45 | 1.43 | 0.28 | 0.62 |
| 100 | 104.0 | 447 | 36.5 | 0.51 | 1.55 | 0.29 | 0.62 |
| 150 | 105.0 | 527 | 43.8 | 0.53 | 1.88 | 0.31 | 0.64 |
| 200 | 105.0 | 459 | 43.0 | 0.65 | 2.60 | 0.33 | 0.76 |
| *Response* | L* | L** | Q* | L** | L** | L** | L** |

Linear (L) or quadratic (Q) effects significant at P = 0.05(*) or 0.01(**)

Table 2.3 Effect of calcium nitrate fertilizer on plant height, leaf area, neck circumference and leaf and bulb nitrogen (N) and sulphur (S)

| Calcium nitrate applied ($kg \cdot ha^{-1}$) | Plant height (mm) | Leaf area ($cm^2$) | Neck circumfe-rence (mm) | Leaf tissue N $g \cdot kg^{-1}$ | Bulb tissue N $g \cdot kg^{-1}$ | Leaf Tissue S $g \cdot kg^{-1}$ | Bulb tissue S $g \cdot kg^{-1}$ |
|---|---|---|---|---|---|---|---|
| | | | *54 days after planting* | | | | |
| 0 | 41.1 | 56.9 | 25.4 | 1.58 | 0.23 | 0.70 | 0.17 |
| 50 | 43.9 | 70.4 | 27.4 | 1.59 | 0.53 | 0.34 | 0.11 |
| 100 | 44.9 | 71.1 | 27.5 | 1.70 | 0.54 | 0.27 | 0.09 |
| 150 | 46.1 | 86.9 | 32.0 | 1.77 | 0.62 | 0.18 | 0.01 |
| 200 | 46.9 | 94.1 | 33.6 | 2.03 | 0.68 | 0.10 | 0.00 |
| *Response* | L** | L** | L** | L** | L** | L** | L** |
| | | | *82 days after planting* | | | | |
| 0 | 45.9 | 102.4 | 31.2 | 2.24 | 0.53 | 0.68 | 0.25 |
| 50 | 48.2 | 129.0 | 33.1 | 2.42 | 0.53 | 0.47 | 0.21 |
| 100 | 50.5 | 145.8 | 35.8 | 2.73 | 0.58 | 0.40 | 0.19 |
| 150 | 51.3 | 153.3 | 38.8 | 2.79 | 0.71 | 0.22 | 0.03 |
| 200 | 54.8 | 200.1 | 43.1 | 2.80 | 0.71 | 0.09 | 0.00 |
| *Response* | L** | L** | L** | L** | L** | L** | L** |
| | | | *112 days after planting* | | | | |
| 0 | 63.9 | 245.2 | 44.9 | 1.89 | 0.98 | 0.54 | 0.28 |
| 50 | 65.5 | 281.8 | 48.0 | 2.49 | 1.05 | 0.41 | 0.25 |
| 100 | 70.5 | 286.2 | 50.0 | 2.66 | 1.13 | 0.33 | 0.22 |
| 150 | 73.2 | 355.8 | 51.5 | 2.82 | 1.19 | 0.22 | 0.09 |
| 200 | 74.2 | 375.9 | 53.9 | 3.36 | 1.22 | 0.09 | 0.06 |
| *Response* | L** | L** | L** | L** | L** | L** | L** |
| | | | *140 days after planting* | | | | |
| 0 | 857 | 313.2 | 52.1 | 1.49 | 1.27 | 0.52 | 0.32 |
| 50 | 88.5 | 353.4 | 53.6 | 1.61 | 1.58 | 0.37 | 0.29 |
| 100 | 93.0 | 383.3 | 56.3 | 1.81 | 1.83 | 0.22 | 0.26 |
| 150 | 99.5 | 528.7 | 60.7 | 1.82 | 1.94 | 0.16 | 0.22 |
| 200 | 100.9 | 502.1 | 60.9 | 1.82 | 1.98 | 0.08 | 0.17 |
| *Response* | L** | Q** | L** | L** | L** | L** | L** |
| | | | *175 days after planting* | | | | |
| 0 | 85.6 | 306.5 | 30.8 | 0.41 | 0.23 | 0.25 | 0.54 |
| 50 | 106.3 | 456.9 | 41.5 | 0.64 | 1.24 | 0.15 | 0.43 |
| 100 | 107.0 | 546.7 | 41.8 | 0.65 | 1.35 | 0.11 | 0.29 |
| 150 | 110.8 | 540.0 | 45.5 | 0.66 | 1.79 | 0.07 | 0.26 |
| 200 | 112.3 | 612.6 | 47.5 | 0.72 | 2.36 | 0.00 | 0.23 |
| *Response* | L** | L** | L** | L** | L** | L** | L** |

Linear (L) or quadratic (Q) effects significant at P = 0.05(*) or 0.01(**)

At 54 days after planting (DAP), N increased fresh leaf mass than dry leaf mass (Figures 2.1 to 2.4). Fresh leaf mass increased with an increase of ammonium sulphate and calcium nitrate application regardless of the sampling date (Figures 2.1 and 2.2). Application of ammonium sulphate from 0 to 200 kg·ha$^{-1}$ increased fresh leaf mass of the plant grown up to 54, 82, 112, 140 and 175 DAP (Figure 2.1). Application of ammonium sulphate at 200 kg·ha$^{-1}$ and at 175 DAP resulted in 3:1 fresh leaf mass and dry leaf mass. Fresh mass were not declining and the leaves were still green but their tops fell over the side indicating physiological maturity (Figure 3.5). The moisture content of fresh mass minus dry mass was 25.33 g.

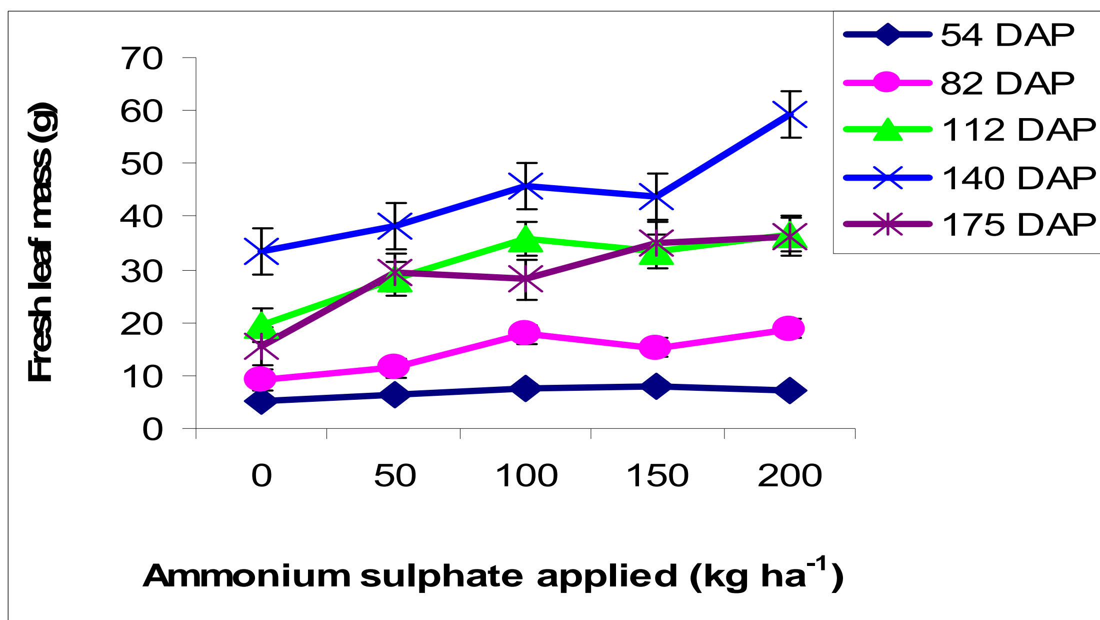


Figure 2.1 Effect of ammonium sulphate on fresh leaf mass of *A. sativum*

The application of calcium nitrate increased fresh leaf mass of the plants harvested on 54, 82, 112, 140 and 175 DAP (Figure 2.2). The greatest leaf mass were produced at 150 and 200 kg·ha$^{-1}$. Untreated plants produced the lowest mass from all sampling dates.

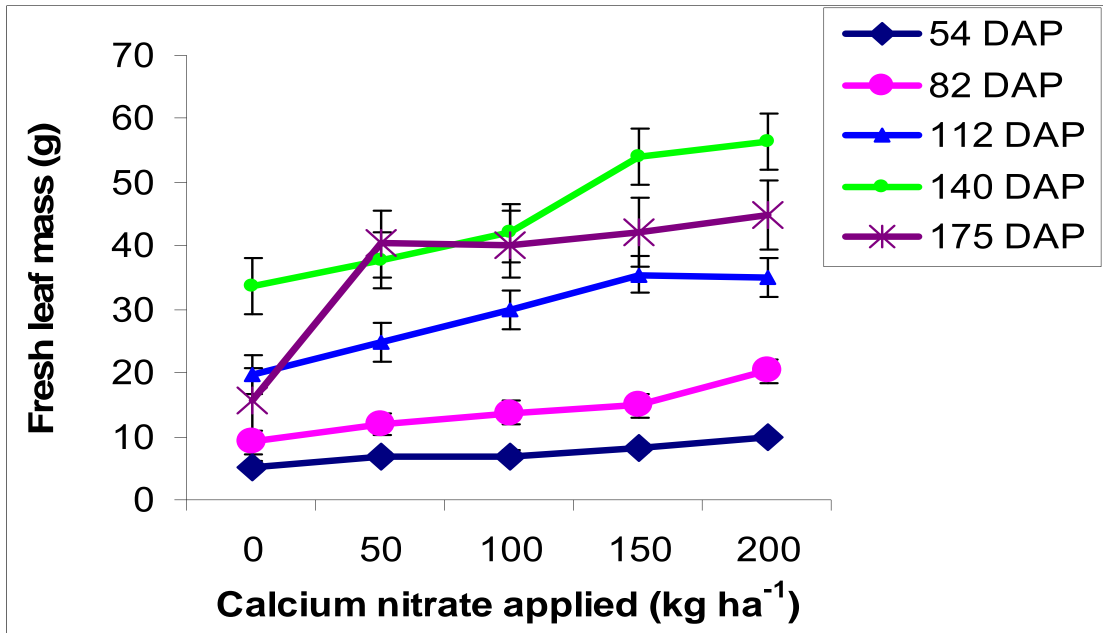


Figure 2.2 Effect of calcium nitrate on fresh leaf mass of *A. sativum*

Dry leaf mass increased in response to an increase in applied ammonium sulphate and calcium nitrate during the growing period (Figures 2.3 and 2.4). Increasing ammonium sulphate application from 0 to 200 kg·ha$^{-1}$ resulted in an increase in dry leaf mass of the plants grown up to 54, 82, 112, 140 and 175 DAP (Figure 2.3).

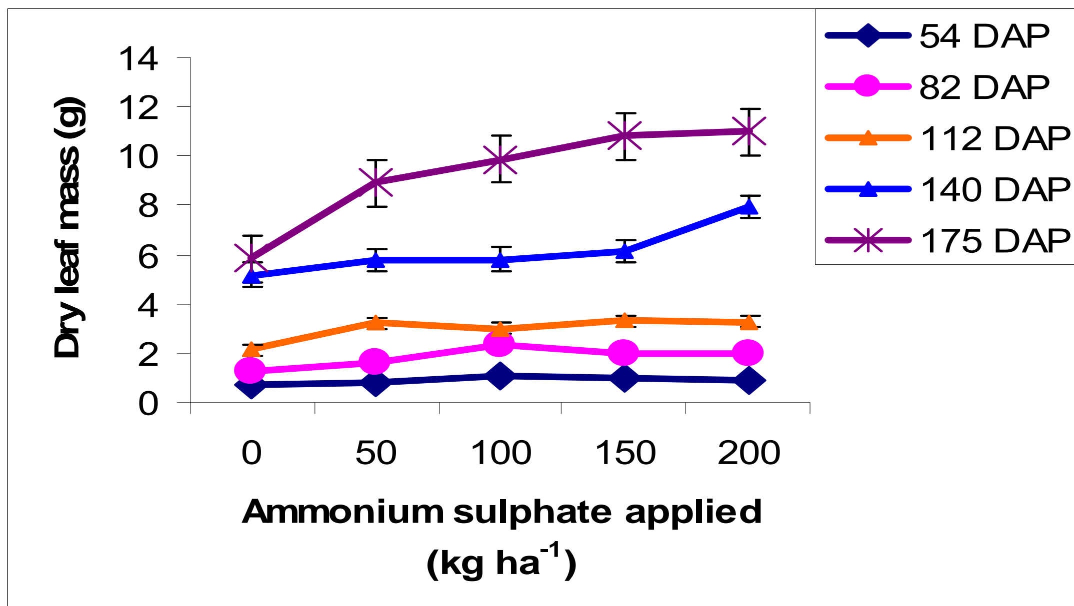


Figure 2.3 Effect of ammonium sulphate on dry leaf mass of *A. sativum*

Plants were harvested at 54, 82 and 112 days after planting during the vegetative stages and at 140 DAP during translocation stage and last harvest were at maturity 175 DAP. Dry leaf mass increased in response to an increase of calcium nitrate applications regardless of the sampling date (Figures 2.4).

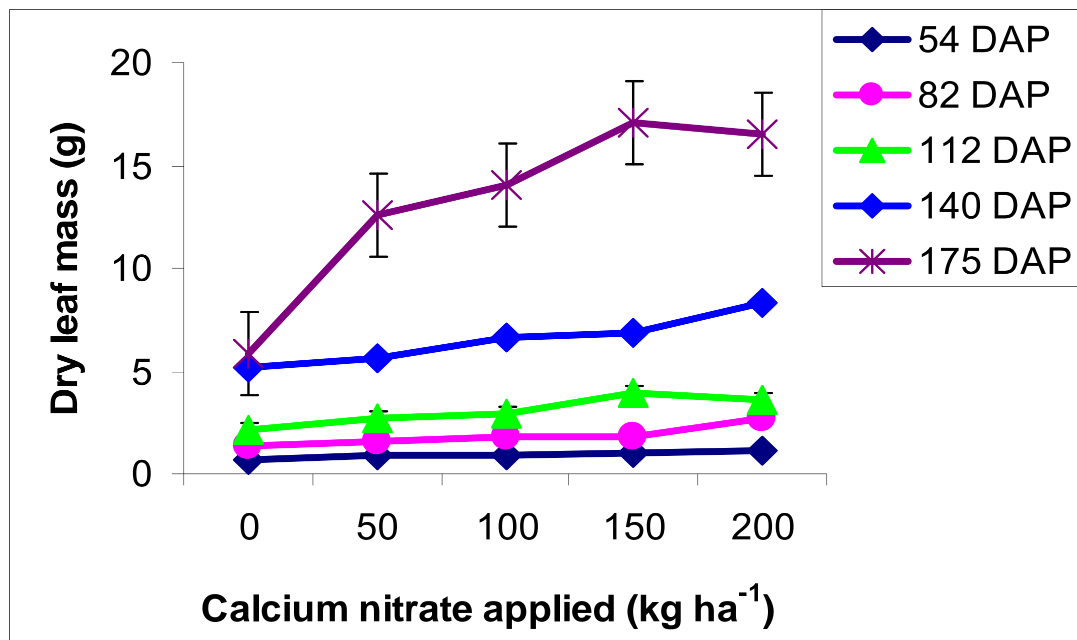


Figure 2.4 Effect of calcium nitrate on dry leaf mass of *A. sativum*

### 2.3.2 Fresh and dry bulb mass

At the beginning of the growing season, fresh bulb mass was low because plants were still at a sprouting stage (54 DAP). At 140 DAP fresh bulb mass increased with increasing ammonium sulphate and calcium nitrate fertilization. A similar tendency was noticed during bulb development. Cloves were clearly formed and visible from this stage of bulb development, and plants underwent a process of translocation that ended at the plant senescence stage. Translocation stage was the last and most important stage, because leaf photosynthates moved from leaves to the bulb (Bertoni, Morard & Liorens, 1992). During harvesting time, bulbs were fully formed and had increased mass with increasing ammonium sulphate or calcium nitrate application. Dry bulb mass of *A. sativum* sampled 54, 82, 112, 140 and 175 DAP increased

with an increase of ammonium sulphate and calcium nitrate fertilization (Table 2.4). At first, plants increased in growth rate and dry mass of leaves than bulbs. The maximum bulb dry mass of 32.03 g (150 kg·ha$^{-1}$) calcium nitrate. The different between the lowest and the highest was 20.01 g.

Table 2.4 Effect of ammonium sulphate and calcium nitrate levels on the bulb dry mass of *A. sativum* harvested at different harvesting dates after planting

| | Days after planting | | | | |
|---|---|---|---|---|---|
| Level | 54 | 82 | 112 | 140 | 175 |
| | | | Ammonium sulphate | | |
| 0 | 0.47b | 0.53b | 0.67b | 1.60b | 10.02c |
| 50 | 0.54a | 0.77a | 1.14a | 2.08a | 14.06bc |
| 100 | 0.55a | 0.83a | 1.14a | 2.20a | 16.10bc |
| 150 | 0.57a | 0.84a | 1.22a | 2.18a | 20.12b |
| 200 | 0.67a | 0.91a | 1.27a | 2.67a | 24.23ab |
| | | | Calcium nitrate | | |
| 0 | 0.47b | 0.53b | 0.67b | 1.60b | 10.02c |
| 50 | 0.49a | 0.72ab | 0.98a | 1.97a | 23.40ab |
| 100 | 0.52a | 0.73ab | 1.17a | 2.03a | 28.73a |
| 150 | 0.57a | 0.85a | 1.26a | 2.23a | 32.03a |
| 200 | 0.51a | 1.07a | 1.26a | 3.13a | 28.21a |
| Grand mean | 0.54 | 0.80 | 1.12 | 2.17 | 22.59 |
| LSD | 0.18 | 0.30 | 0.40 | 1.92 | 6.39 |

Means within the same columns followed by the same letter are not significantly different at 5% level

### 2.3.3 Leaf and bulb tissue nitrogen

Leaf tissue N increased linearly with increasing ammonium sulphate and calcium nitrate fertilization. As ammonium sulphate and calcium nitrate applications increased from 0 to 200 kg·ha$^{-1}$, leaf tissue N increased from 1.58 to 3.03 g·kg$^{-1}$ until 140 DAP. Thereafter, leaf tissue N decreased to 0.65

$g \cdot kg^{-1}$. Bulb tissue N increased from 0.42 to 2.60 $g \cdot kg^{-1}$ at 175 DAP as N applied increased (Tables 2.2 and 2.3). Widders (1989) and Kakara *et al.* (2002) suggested that leaf tissue N is important for plant establishment after plant emergence (sprouting stage) at early vegetative stage as it influences vigorous growth.

### 2.3.4 Leaf and bulb tissue sulphur

The analysis of plant materials using elements present is another type of approach in determining nutrient availability in plants (Mengel & Kingsly, 2001). From the results obtained, leaf tissue S increased linearly with increasing ammonium sulphate fertilization (Table 2.2). As ammonium sulphate application increased from 0 to 200 $kg \cdot ha^{-1}$, leaf tissue S increased from 0.70 to 0.91 $g \cdot kg^{-1}$ at 54 DAP. Thereafter, leaf tissue S produced at 140 and 175 DAP were lower than those one produced at 54, 82 and 112 DAP (Table 2.2). The obtained results confirm the report of Bradford & Hornbacher (1988) that the sulphur leaf concentration increased during the early plant development and decreased as bulbing progressed to maturity, plants tops were old and started to fell over. Bulb tissue S increased from 0.54 to 0.76 $g \cdot kg^{-1}$ at 175 DAP (Table 2.2). Little percentage S was obtained at 54 and 82 DAP on *A. sativum* leaves that were treated with 150 and 200 $kg \cdot ha^{-1}$ N supplied from calcium nitrate and decreasing from 0.70 to 0.10 $g \cdot kg^{-1}$ (Table 2.3). No percentage S was obtained at 54 and 82 DAP on *A. sativum* bulbs that were treated with 200 $kg \cdot ha^{-1}$ N supplied from calcium nitrate, decreasing from 0.54 to 0.23 $g \cdot kg^{-1}$ due to the fact that plants was not supplied with sulphur fertilizer.

### 2.3.5 Effects of nitrogen nutrition on *A. sativum* bulb growth

Nitrogen is important for *A. sativum* growth (Bertoni *et al.,* 1992) as it is often applied in greatest quantity compared to other fertilizer elements. Results in Table 2.5 indicate that calcium nitrate applied at 150 and 200 $kg \cdot ha^{-1}$ produced the greatest neck circumference while ammonium sulphate at 150 and 200 $kg \cdot ha^{-1}$ produced lower neck circumferences. The present results

were in agreement with those of Brewster & Butler (1989) and Potgieter (2006), where a thick neck circumference was reported to have increased as a result of an increase in calcium nitrate application at 200 kg·ha$^{-1}$. The highest bulb circumference was produced at 200 kg·ha$^{-1}$ ammonium sulphate application but it was not significantly different from the 150 kg·ha$^{-1}$ calcium nitrate treatment. Calcium nitrate applied at 150 kg·ha$^{-1}$ produced the greatest bulb mass, while bulb mass produced from plants that were treated with ammonium sulphate were significantly lower. At final harvest (175 DAP), bulb mass increased considerably with an increase in N application. Plants that were treated with 100, 150 and 200 kg·ha$^{-1}$ calcium nitrate produced the greatest number of cloves and were not significantly different from plants treated with 200 kg·ha$^{-1}$ ammonium sulphate. The highest marketable yield was produced at 200 kg·ha$^{-1}$ ammonium sulphate (27.23 t·ha$^{-1}$), which was not significantly different from those of the 150 and 200 kg·ha$^{-1}$ calcium nitrate treatments.

The development of cloves on the stem (Figure 2.5) occurred when 200 kg·ha$^{-1}$ N as calcium nitrate was applied. About 20% of the plants that were treated with 200 kg·ha$^{-1}$ N in the form of calcium nitrate resulted in physiological disorders. Plants that were treated with 0, 50, 100 and 150 kg·ha$^{-1}$ N in the form of calcium nitrate and 0 to 200 kg·ha$^{-1}$ N in the form of ammonium sulphate did not show the disorder.

Table 2.5 Effect of ammonium sulphate and calcium nitrate fertilizer on neck and bulb circumference, bulb mass, bulb cloves and marketable yield of *A. sativum*

| N levels ($kg\cdot ha^{-1}$) | Neck circumference (mm) | Bulb circumference (mm) | Bulb mass (g) | Number of cloves per bulb | Marketable yield ($t\cdot ha^{-1}$) |
|---|---|---|---|---|---|
| | | Calcium nitrate fertilizer | | | |
| 0 | 30.75 d | 123.50f | 43.70d | 19.08d | 19.40b |
| 50 | 41.50abc | 179.50c | 116.10ab | 29.88a | 21.45ab |
| 100 | 41.75abc | 184.20bc | 117.70ab | 34.38a | 22.00ab |
| 150 | 45.75a | 196.20a | 128.80a | 34.58a | 24.56a |
| 200 | 47.50a | 186.80ab | 125.20a | 34.38a | 22.67ab |
| | | Ammonium sulphate fertilizer | | | |
| 0 | 30.75d | 123.50f | 43.70d | 19.08d | 19.40b |
| 50 | 35.50cd | 159.20e | 79.90c | 24.67c | 19.46ab |
| 100 | 36.50bcd | 163.50de | 82.70c | 27.92bc | 23.16ab |
| 150 | 43.00ab | 183.00bc | 101.50b | 29.83b | 24.62a |
| 200 | 43.75ab | 196.80a | 105.20b | 33.54a | 27.23a |
| Grand mean | 41.20 | 169.60 | 64.88 | 30.00 | 22.73 |
| LSD | 5.77 | 10.15 | 9.94 | 2.95 | 3.65 |

Means within the same columns followed by the same letter are not significantly different at 5% level

Figure 2.5 Development of cloves on the stem of *A. sativum* (A- cloves)

## 2.4 CONCLUSIONS

It is important for growers to determine the best nitrogen source and the suitable nitrogen level that will result in best plant yield and quality. Both ammonium sulphate and calcium nitrate improved plant height and fresh leaf mass of *A. sativum* plants. The highest percentage leaf tissue N was obtained from *A. sativum* leaves in plants that were treated with 200 $kg \cdot ha^{-1}$ N supplied from calcium nitrate at 112 DAP. A higher percentage S was obtained at 82 DAP in *A. sativum* leaves which were treated with 200 $kg \cdot ha^{-1}$ N supplied from ammonium sulphate. There were no percentage S obtained at 54 and 82 DAP in *A. sativum* bulbs that were treated with 200 $kg \cdot ha^{-1}$ N supplied from calcium nitrate.

*A. sativum* plants treated with ammonium sulphate produced the highest yield of 27 $t \cdot ha^{-1}$ at 200 $kg \cdot ha^{-1}$ N. The highest yield was obtained from calcium nitrate that was from plants treated with 150 $kg \cdot ha^{-1}$ N which resulted in a yield of 24 $t \cdot ha^{-1}$. Therefore, ammonium sulphate can be recommended as the best nitrogen source over calcium nitrate for maximizing bulb yield.

## 2.5 SUMMARY

The objectives of this study were to determine the ideal ammonium sulphate and calcium nitrate levels for optimal *A. sativum* plant growth and bulb yield, and to determine nitrogen and sulphur accumulation at different stages of plant development. The experiment was carried out in eight months, from April to October 2006. The main parameters that were considered included plant height, leaf area, fresh and dry leaf mass, leaf and bulb N, and leaf and bulb S. These data plants were destructively harvested at different sampling dates from 54, 82, 112, 140 to 175 days after planting (DAP).

The effects of ammonium sulphate and calcium nitrate fertilizer on neck and bulb circumference and bulb mass were significant (Table 2.3). Highest percentage leaf N was obtained from *A. sativum* leaves harvested at 140 DAP and were treated with 150 and 200 $kg \cdot ha^{-1}$ N supplied from ammonium sulphate. Highest percentage bulb N was obtained at 200 $kg \cdot ha^{-1}$ N supplied from ammonium sulphate in plants that were harvested at 175 DAP. The highest percentage S was obtained from *A. sativum* leaves harvested from plants produced with 200 $kg \cdot ha^{-1}$ N supplied from ammonium sulphate and at 82 and 175 DAP. *A. sativum* plants produced with the calcium nitrate treatment resulted in the highest percentage leaf N when harvested at 112 DAP and the highest percentage bulb N when harvested at the last harvesting date (175 DAP).

The highest bulb yield from plants produced with calcium nitrate was 24 $t \cdot ha^{-1}$ at 150 $kg \cdot ha^{-1}$ N, while plants produced with ammonium sulphate gave the highest yield of 27 $t \cdot ha^{-1}$ with 200 $kg \cdot ha^{-1}$ N. Therefore, ammonium sulphate is a recommended source of nitrogen fertilizer in terms of maximizing *A. sativum* bulb yield.

# CHAPTER 3

# EFFECTS OF *ALLIUM SATIVUM* EXTRACTS AGAINST PLANT PATHOGENS *ALTERNARIA SOLANI* AND *SCLEROTIUM ROLFSII*

## 3.1 INTRODUCTION

Egyptian white garlic (*Allium sativum)* can be considered as a vegetable and as a herbal crop throughout the world including South Africa. In South Africa, it is mostly produced by small scale and commercial farmers in Limpopo, Gauteng and Free State Provinces. *A. sativum* extracts have historically been used to treat several human ailments such as flu, fever, colds, tuberculosis, cancer, cholesterol, blood pressure, diarrhea due to intestinal bacterial imbalance, urinary infections and asthma (Sivam, 2001; Pamplona-Roger & Malaxetxebarria, 2004).

There is little information in literature about the use of *A. sativum* plant extracts as a source of antifungal agents (Yoshida, Kasuga, Hayashi, Ushiroguchi, Matsuura & Nakagana, 1987) that could provide a safe and easily biodegradable alternative to more commonly used synthetic fungicides (Motsei, Lindsey, Van Staden & Jager, 2003; Lindsey & Van Staden, 2004; Nteso & Pretorius, 2006).

The aim of this study was to determine the influence of ammonium sulphate and calcium nitrate nutrition on the bioactivity of *A. sativum* plants. This was conducted by screening extracts of plants that had been harvested at five different stages of plant growth, and screening them against plant pathogens *Alternaria solani* and *Sclerotium rolfsii*.

## 3.2 MATERIALS AND METHODS

### 3.2.1 Plant materials

The plant materials of *A. sativum* that were collected from the first field experiment (Chapter 2) were used for screening in a laboratory. Plant materials were screened against *S. rolfsii* and *A. solani* plant pathogens.
*A. sativum* plant material was harvested at 54, 82, 112, 140 and 175 days after planting (DAP). Harvested plants were divided into aerial parts and underground parts, and their fresh mass was determined. The plant material was then dried in an air-forced oven at 40 ºC for two weeks, then the dry mass was taken. The dry aerial and underground plant parts were milled separately.

### 3.2.2 Crude extract

Plant extracts were prepared from leaves and bulbs according to the method described by Rios, Recio & Villar (1988). Samples (2g) of powdered plant material were extracted with methanol. Bottles were placed on a shaker for 24 hours, and thereafter they were filtered under vacuum through Whatman No.1 filter papers. The filtrates were poured into 65 mm petri dishes which were left open to allow the methanol to evaporate over night. After drying, the plant extracts were stored at –20 ºC.

Antifungal activity was qualitatively evaluated by means of the agar plate diffusion assay technique (Rios *et al.*, 1988). Malt agar was used to prepare mother cultures of two plant pathogens (*S. rolfsii* and *A. solani*) obtained from the Plant Protection Research Institute of the Agricultural Research Council (ARC) in Pretoria, South Africa. Two mother cultures were prepared for each fungus, with one being a working culture and the other a back-up culture stored in a refrigerator at 4-10 ºC.

### 3.2.3 Experimental design and treatment details

The two plant pathogenic fungi were cultured on 0.05% malt agar that was prepared according to the instructions from Merck (Nteso & Pretorius, 2006). The malt agar solution was autoclaved for 20 minutes at 125ºC and then cooled to 45 ºC in a water bath. An amount of 33% (m/v) streptomycin solution was added to the basal medium to control bacterial growth. A methanol extract was dissolved in 0.3 ml sterile distilled water to make a concentration of 0.45 $mg \cdot mL^{-1}$ and amended in the agar solution to yield a final concentration of 60.45 $mg \cdot mL^{-1}$. Thereafter, the medium was poured into 65 mm petri dishes and allowed to set under sterile conditions on a laminar flow cabinet. The centre of each petri dish was inoculated with a 6 mm diameter plug obtained from the mother culture. A petri dish containing only the basal medium served as a control. Each treatment was replicated four times. The plates were incubated for 2 days at 25 ºC in a growth cabinet

### 3.2.4 Statistical analysis

Results were statistically analyzed using GenStat (2005). Differences between treatments were tested by means of analysis of variance (ANOVA). The treatment means were separated using LSD test at the 5% level of significance.

## 3.3 RESULTS AND DISCUSSION

### 3.3.1 Effects of *A. sativum* leaf extracts against *A. solani* and *S. rolfsii*

The inhibition of plant pathogens demonstrated that extracts from this plant could be utilized to control fungal infections as reported previously by Nteso & Pretorius (2006). Leaf extracts that were obtained from *A. sativum* plants that were harvested at 54, 82, 112, 140 and 175 (DAP) inhibited the growth of *S. rolfsii* and *A. solani* (Figure 3.1). Results found at 54 and 175 DAP were significantly between *S. rolfsii* and *A. solani* while at 82, 112 and 140 DAP not significant differences were found.

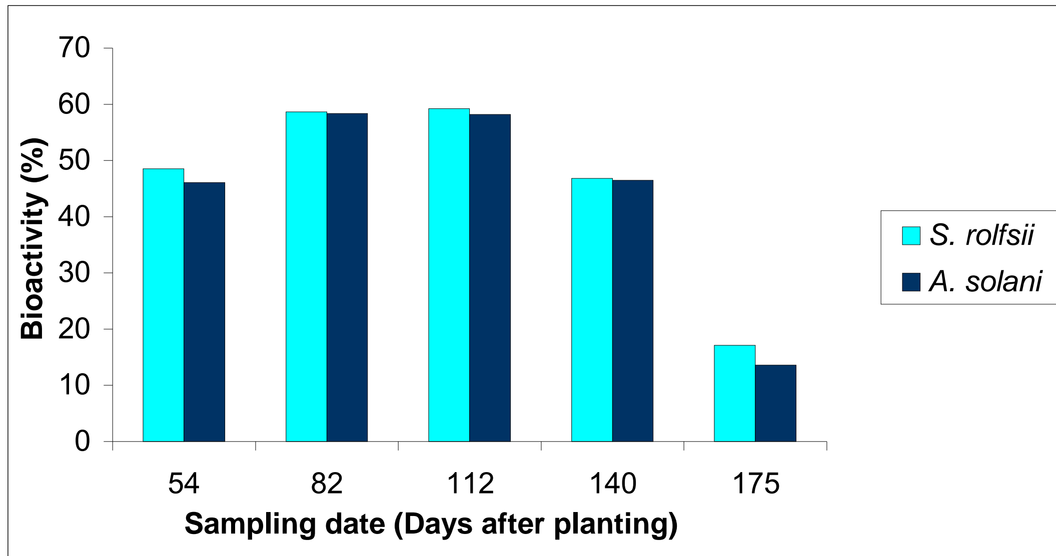


Figure 3.1 Effects of bioactivity on the growth of *S. rolfsii* and *A. solani* at different sampling dates of *A. sativum* leaves

Generally, leaf bioactivity increased when the number of days and nitrogen levels increased (Figure 3.2). The highest bioactivity was found from leaves harvested at 112 DAP, but the results were not significantly different from those obtained at 82 DAP when 200 $kg \cdot ha^{-1}$ammonium sulphate was applied. At 82 and 112 DAP plant leaves had grown to an optimal size. At this stage, it was observed that more sulphate was stored in the leaves than in the bulbs (Chapter 2). At 140 DAP leaf bioactivity percentage started to decrease. *A. sativum* leaves produced the lowest bioactivity at 175 DAP.

Leaf extracts of *A. sativum* plants that were treated with calcium nitrate fertilizer demonstrated low bioactivity (Figure 3.3) when compared to plants that were treated with ammonium sulphate. Ammonium sulphate may improve the *A. sativum* bioactivity due to sulphur element that was applied to the plant (Van den Heever, 2006). After 112 DAP, leaf bioactivity decreased significantly regardless of the nitrogen source applied. There were no-linear regression for *A. sativum* leaf between sampling dates and fertilizer level for *A. solani* and *S. rolfsii*.

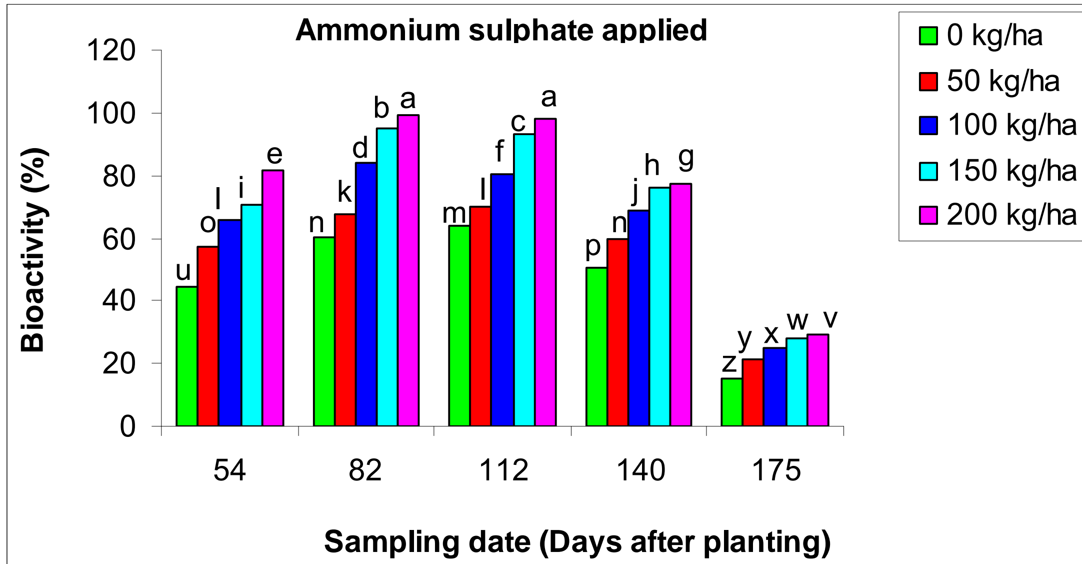


Figure 3.2 Effects of ammonium sulphate (kg·ha$^{-1}$) on the bioactivity of *A. sativum* leaves at different sampling dates (DAP)

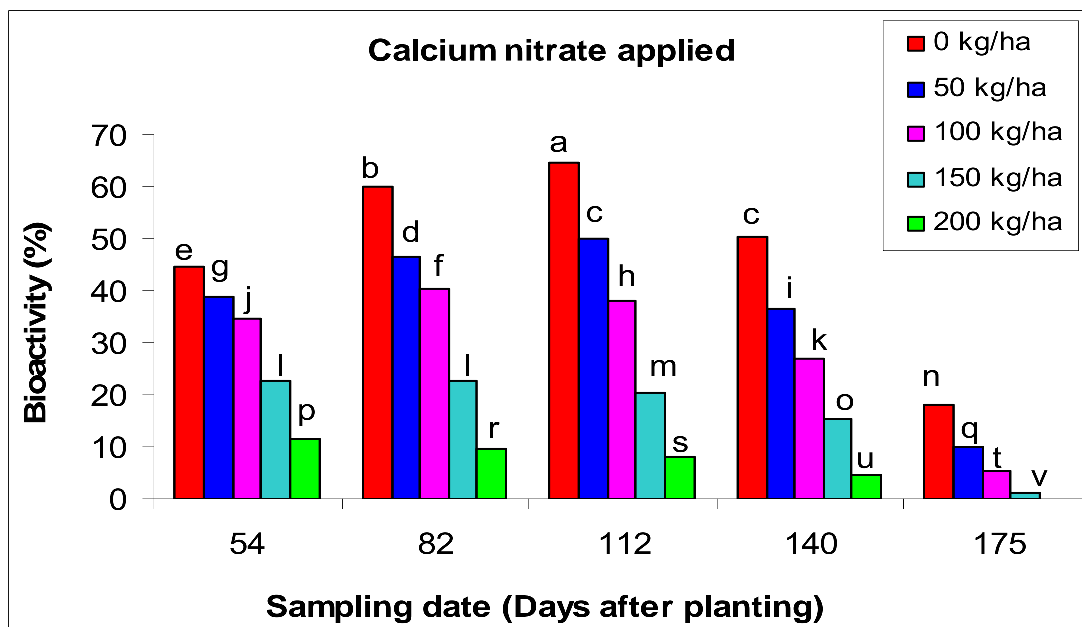


Figure 3.3 Effects of calcium nitrate (kg·ha$^{-1}$) on the bioactivity of *A. sativum* leaves at different sampling dates (DAP)

*A. sativum* leaves can be harvested between 82 and 112 DAP when leaves reach their optimum development stage (Figure 3.4).

Figure 3.4 *A. sativum* plants at vegetative stage (112 DAP) of plant growth

*A. sativum* leaves that were harvested at 175 DAP produced the lowest bioactivity. At this period, leaves were too old and stems started to fall over and bulbs had reached their maturity stage (Figure 3.5).

Figure 3.5 *A. sativum* plants at maturity stage (175 DAP) of plant growth

The inhibition of plant pathogens shows that plant extracts harvested at early stage of plant growth were active against plant pathogens as reported previously by Nteso & Pretorius (2006).

### 3.3.2 Effects of *A. sativum* bulb extract against *A. solani* and *S. rolfsii*

It was important to determine bioactivity on *A. sativum* leaves and bulbs at different stages of plant growth. Bulb extracts that were obtained from *A. sativum* bulbs that were harvested at 54, 82, 112, 140 and 175 (DAP) inhibited the growth of *S. rolfsii* and *A. solani* (Figure 3.6). The bioactivity increased as the harvesting date increased.

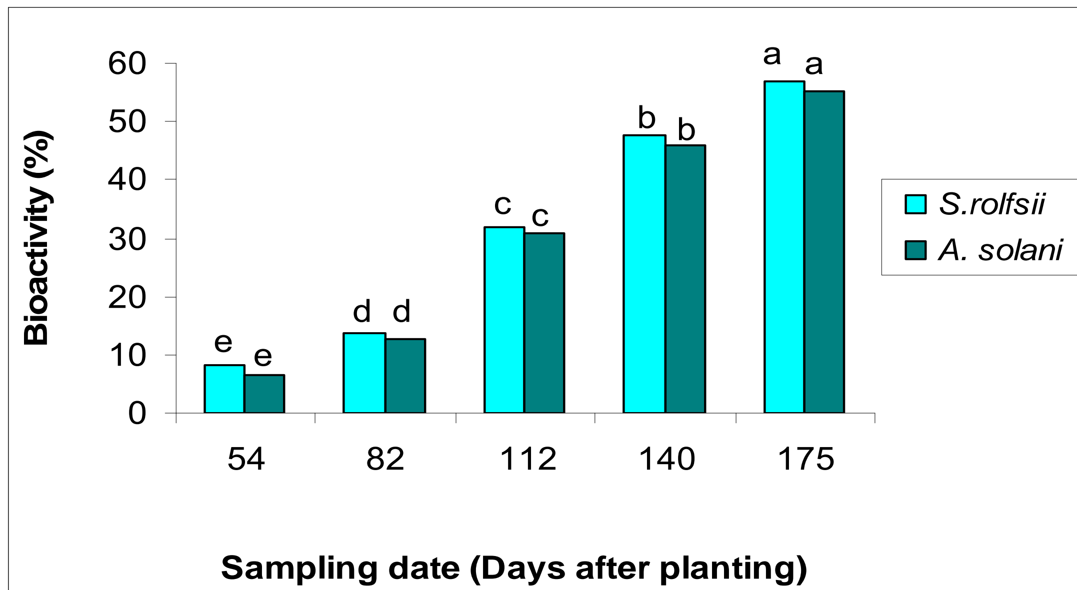


Figure 3.6 Effects of bioactivity (%) on the growth of *S. rolfsii* and *A. solani* at different sampling dates of *A. sativum* bulbs

Figure 3.6 shows that *A. sativum* bulb extracts produced very low bioactivity percentage at 54 DAP, regardless of the amount of fertilizer applied to the plant. This could be attributed to plants using the applied fertilizer for vegetative growth at an early stage of bulb formation.

The highest percentage inhibition against plant pathogens was found at 200 $kg{\cdot}ha^{-1}$ ammonium sulphate, whereas the results were not significantly different from 100 to 150 $kg{\cdot}ha^{-1}$ at 175 DAP (Figure 3.7). There were no-linear regression for *A. sativum* bulb between sampling dates and fertilizer level for *A. solani* and *S. rolfsii*.

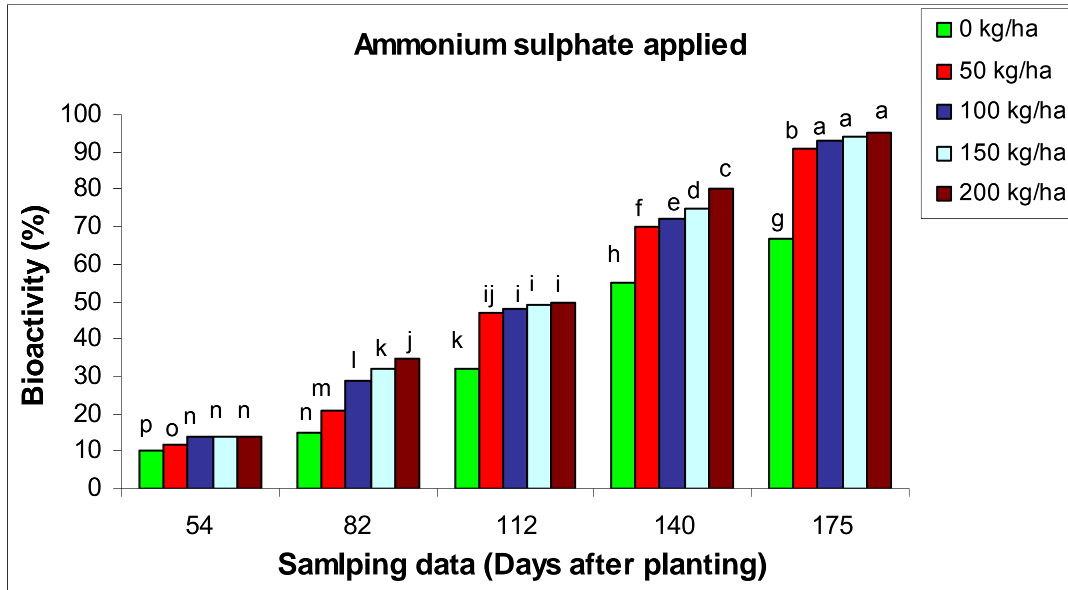


Figure 3.7 Effects of ammonium sulphate on the bioactivity (%) of *A. sativum* bulbs at different sampling dates (DAP)

The more calcium nitrate was applied to the plants, the more significant was the lowering of the bioactivity of the bulbs compared to the control. The results of Figure 3.8 indicates that at 54 and 82 DAP plants that were treated with 150 and 200 kg·ha$^{-1}$ calcium nitrate did not have any bioactivity.

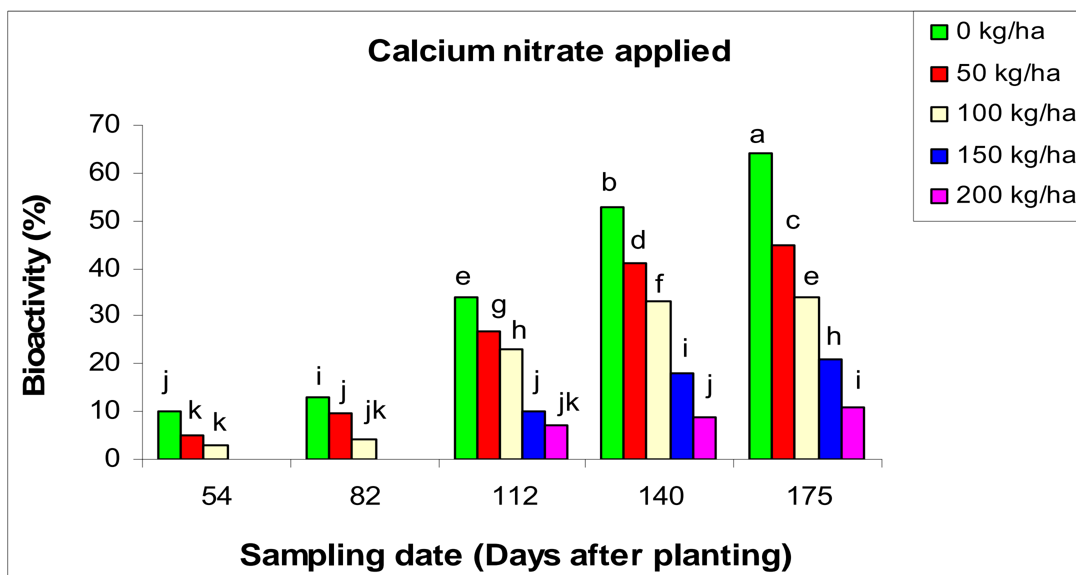


Figure 3.8 Effects of calcium nitrate on the bioactivity (%) of *A. sativum* bulbs at different sampling dates (DAP)

Plants that received ammonium sulphate fertilizer increased in bioactivity percentage with each increase in the level of ammonium sulphate applied, while plants that received calcium nitrate fertilizer had a low bioactivity percentage.

The mean average bioactivity of *A. sativum* bulb extracts increased simultaneously with an increase in the number of days after planting regardless of the nitrogen source used (Figure 3.9). Plants that received ammonium sulphate produced greater bioactivity than calcium nitrate grown plants. The results of both nitrogen sources were significantly different from each other.

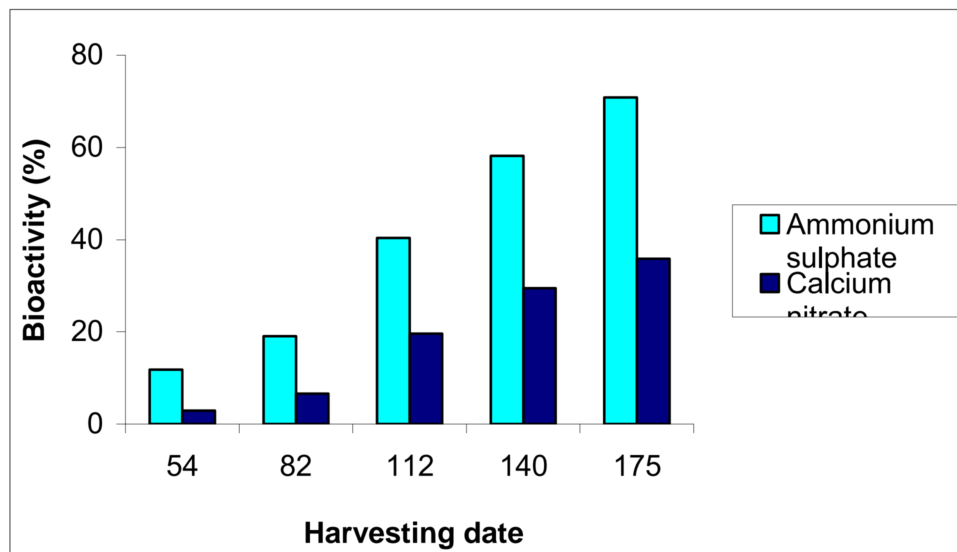


Figure 3.9 Effects of nitrogen nutrition and harvesting date on bioactivity percentage of *A. sativum* bulbs

### 3.3.3 Bioactive phytochemicals and functional foods

*A. sativum* (bulb or clove) is one of many multi-purpose plants. It has the potential of preventing diseases and therefore has health-promoting properties (Bakri & Douglas, 2005). It is an important common food and plays a medicinal role, which has a broad spectrum of antimicrobial activity (Williams & Lamprecht, 2007). *A. sativum* is also one of the functional foods that are commonly used world-wide. It is a food for specific health use and is

defined as food derived from natural occurring substances that can regulate a particular body process when consumed as part of a daily diet (Narasinga, 2003). It is also classified as an enhancer of the body's immune system; preventer of diabetes and heart diseases; hypocholesterolaemic agent; promoter of digestion and absorption and retardant of ageing (Narasinga, 2003; Corzo-Martinez, Corzo & Villamiel, 2007).

The nutritional value of this crop was also affected by the application of large amounts of fertilizers (Table 3.1). The more fertilizer was applied, the lower was the percentage fat in the mature *A. sativum* bulb, regardless of the type of nitrogen fertilizer applied to the plant. When the amount of nitrogen fertilizer was increased, the percentage dietary fibre also increased. Greater increase in vitamin C was found when ammonium sulphate was increased from 0 to 200 $kg \cdot ha^{-1}$. Calcium nitrate increases the amount of vitamin C that was found on *A. sativum* bulb at a lower level when compared to ammonium sulphate fertilizer (Narasinga, 2003).

Table 3.1: Effects of ammonium sulphate and calcium nitrate on fat, dietary fibre and vitamin C in *A. sativum* bulbs

| | Fat (ether extraction) (%) | Dietary fibre (Total) (%) | Vitamin C mg/100 g |
|---|---|---|---|
| Control | 0.23 | 10.61 | 0.36 |
| 50 $kg \cdot ha^{-1}$ $(NH4)_2SO_4$ | 0.26 | 11.02 | 1.34 |
| 50 $kg \cdot ha^{-1}$ $Ca(NO_3)_2$ | 0.37 | 12.75 | 0.61 |
| 100 $kg \cdot ha^{-1}$ $(NH4)_2SO_4$ | 0.00 | 12.76 | 1.50 |
| 100 $kg \cdot ha^{-1}$ $Ca(NO_3)_2$ | 0.00 | 12.94 | 0.77 |
| 150 $kg \cdot ha^{-1}$ $(NH4)_2SO_4$ | 0.00 | 16.25 | 1.59 |
| 150 $kg \cdot ha^{-1}$ $Ca(NO_3)_2$ | 0.00 | 16.30 | 0.77 |
| 200 $kg \cdot ha^{-1}$ $(NH4)_2SO_4$ | 0.00 | 18.94 | 1.85 |
| 200 $kg \cdot ha^{-1}$ $Ca(NO_3)_2$ | 0.00 | 17.07 | 0.81 |

## 3.4 CONCLUSIONS

Results obtained from this study confirmed the correlation between sampling date and percentage bioactivity of *A. sativum* leaves and bulb parts. Ammonium sulphate improved leaf bioactivity and the highest bioactivity was obtained at 82 and 112 DAP. After 112 DAP leaf bioactivity started to decline, and by then leaves had grown older and started to die off. Bioactivity percentage was higher from plants produced with ammonium sulphate than those produced with calcium nitrate. Bulbs produced very low bioactivity during sprouting and vegetative stages of plant growth, regardless of nitrogen level and source. During translocation and maturity stages, *A. sativum* bulbs developed with ammonium sulphate treatment produced greater bioactivity than bulbs developed from calcium nitrate treatment. There was also a negative relationship between calcium nitrate applied and bioactivity. Therefore, only ammonium sulphate can be recommended as a better source of nitrogen fertilizer. Findings of this study indicate that calcium nitrate is not recommended since it failed to improve the medicinal properties of *A. sativum* plant.

## 3.5 SUMMARY

The study was conducted to determine the influence of ammonium sulphate and calcium nitrate nutrition on the bioactivity of *A. sativum* plants. It was achieved by screening extracts of plants that had been harvested at five different stages of plant growth, against plant pathogens *A. solani* and *S. rolfsii*. The experiment was conducted in the field at the Experimental Farm of the Agricultural Research Council's Vegetable and Ornamental Plant Institute (ARC-VOPI) at Roodeplaat, Pretoria.

Screenings were made from separate leaves and bulbs of *A. sativum* plant extracts against *A. solani* and *S. rolfsii*. *A. sativum* plants that received higher amounts of ammonium sulphate (100, 150 and 200 $kg \cdot ha^{-1}$) had significantly higher yield as compared to plants that received the lowest level (50 $kg \cdot ha^{-1}$). When plants are still young (54 DAP), ammonium sulphate is not

recommended to improve bioactivity since plants are still developing roots and unable to utilize much of the fertilizer supplied to them.

These results suggest that *A. sativum* extracts from both leaves and bulbs of plants which were supplied with ammonium sulphate can be used as a cost effective alternative to synthetic fungicides to increase the control of fungal pathogens. *A. sativum* plants are superior sources of antifungals for the treatment of both *A. solani* and *S. rolfsii* infections.

# CHAPTER 4

# EFFECT OF NITROGEN NUTRITION ON GROWTH AND PRODUCTIVITY OF WILD GARLIC (*TULBAGHIA VIOLACEA* HARV.)

## 4.1 INTRODUCTION

*Tulbaghia violacea* has been used for centuries as a traditional medicinal plant against various infections. There are also a few studies that have been conducted on the bioactivity of *T. violacea* crop. Besides being commonly used by South African rural communities as a medicine (Van Wyk *et al*., 1997), it is also used in landscaping areas due to its attractive summer flowers (Harris, 2004). However, *T. violacea* crop is an indigenous and underutilised crop, so there is no or little information about fertilization programme of this crop.

Fertilizer management is one of the important management factors that may contribute much to the *allium* yield. Fertilization requirements of this plant involve nitrogen (N) and sulphur (S) as the most important elements required for plant growth (Clark, 1997). Clark (1997) reported that the application of these two elements affects the vegetative growth and reproductive stages of many plants. Nitrogen and sulphur are important nutrient element that play important role on bulb formation, elongation, skin color development and pungency of onion (Mozumder, Moniruzzaman, & Halim, 2007). *T. violacea* has a wide range of secondary compounds that have an impact on its use. Sulphur as a fertilizer influences secondary compounds that are found in this plant and improves pungent odour and flavour of both garlic and onion plants (Leustek & Saito, 1999). The optimum application of nitrogen to *A. sativum* range from 50 to 160 kg·ha$^{-1}$ Brewster & Butler, 1989), Nitrogen fertilization has already been shown to have a strong influence on plant growth and production. Therefore, there is a need to generate basic agronomic information that will make this medicinal plant more productive and sustainable throughout its growing cycle.

The aim of the present study was to determine the effect of nitrogen sources (ammonium sulphate and calcium nitrate) and their levels on plant growth and yield of *Tulbaghia violacea* plants.

## 4.2 MATERIALS AND METHODS

### 4.2.1 Experimental site

A field experiment was conducted at the Experimental Farm of the Agricultural Research Council - Vegetable and Ornamental Plant Institute (ARC-VOPI) at Roodeplaat, Pretoria. The experiment was conducted from April 2006 to April 2007. A randomized complete block design consisting of nine treatments and four replications was used.

### 4.2.2 Planting materials

Planting materials of *T. violacea* bulbs were obtained from Lifestyle Seed Company based in the Free State Province. Seeds were sown on seedling trays from seeds in March 2005 and transplanted on April 2006.

### 4.2.3 Experimental design and treatment details

Two nitrogen sources (ammonium sulphate and calcium nitrate) were applied at five levels (0, 50, 100, 150 and 200 $kg \cdot ha^{-1}$). Application of treatments to *T. violacea* plants was commenced from the third month after planting. It was applied by means of top dressing at three month intervals (Table 4.1).

Table 4.1 Treatment application rates for nitrogen on *T. violacea* plants at three month intervals

| N-source | Nitrogen applied ($kg{\cdot}ha^{-1}$) | Application interval | | 9 months after emergence ($g{\cdot}plot^{-1}$) | Total amount applied ($g{\cdot}plot^{-1}$) |
|---|---|---|---|---|---|
| | | 3 months after emergence ($g{\cdot}plot^{-1}$) | 6 months after emergence ($g{\cdot}plot^{-1}$) | | |
| Control | 0 | 0 | 0 | 0 | 0 |
| $(NH4)_2SO_4$ | 50 | 6.3 | 7.4 | 7.4 | 21.0 |
| $(NH4)_2SO_4$ | 100 | 10.4 | 15.8 | 15.8 | 42.0 |
| $(NH4)_2SO_4$ | 150 | 15.8 | 23.6 | 23.6 | 63.0 |
| $(NH4)_2SO_4$ | 200 | 21.0 | 31.5 | 31.5 | 84.0 |
| $Ca(NO_3)_2$ | 50 | 7.6 | 11.3 | 11.3 | 30.2 |
| $Ca(NO_3)_2$ | 100 | 15.1 | 22.7 | 22.7 | 60.4 |
| $Ca(NO_3)_2$ | 150 | 22.7 | 34.0 | 34.0 | 90.7 |
| $Ca(NO_3)_2$ | 200 | 30.3 | 45.3 | 45.3 | 120.9 |

Plants per plot were destructively harvested monthly from July 2006 to April 2007. Sampling data was collected for number of leaves, plant height (cm), fresh and dry leaf mass (g), fresh and dry bulb mass (g), number of tillers, leaf area ($cm^2$), number of flowers and yield.

Harvested plants were stored in an oven for two weeks at $40^oC$ to dry-out. The dried samples were divided into two halves, with one-half for chemical analysis and the other half for screening of bioactivity.

#### 4.2.4 Statistical analysis

Collected data were subjected to an analysis of variance using GenStat (2005) programme. Treatments sums of squares were partitioned into linear and quadratic contrasts. Significant differences were taken at 5% level.

## 4.3 RESULTS AND DISCUSSION

### 4.3.1 Growth analysis

The overall data for plant height, number of leaves, new tillers, dry leaf mass, dry bulb mass and leaf area as well as yield of *T. violacea* plants are presented in Table 4.2.

### 4.3.2 Effect of nitrogen on plant height

*T. violacea* plants grow very slowly during the beginning of the growing season. Plants which received 200 kg·ha$^{-1}$ (ammonium sulphate) were the tallest as compared to all other levels, followed by 150, 100, 50 kg·ha$^{-1}$ and the shortest were unfertilized plants (Table 4.2). Plant height ranged from 21.2 to 26.5 cm in response to ammonium sulphate and similar results were obtained for plants treated with calcium nitrate. Harris (2004) reported that plant height of *T. violacea* reach a height of 50 cm tall which was not in this case. Plant height values determined in this study were lower than that obtained by Harris (2004). Tallest plants were obtained on plants harvested in September 2006. The percentage leaf tissue N was from 0.64% untreated plants and 2.85% calcium nitrate and 2.80% ammonium sulphate treated plants (Table 4.3 & 4.4). The lower values could have been may be caused by other factors affecting plant height such as genetic sources, climate, growing season, soil properties and many other agricultural practices (Ozguven, Sener, Orhan, Sekeroglu, Kirpik, Kartal, Pesin & Kaya, 2008) which overrules N content.

Table 4.2. Effect of N-sources on plant height, leaf number, leaf area, number of new tillers, dry leaf mass, dry bulb mass and yield of *T. violacea*

| N applied (kg·ha$^{-1}$) | Plant height (cm) | Leaf no. | Leaf area (m$^2$) | No. of new tillers | Dry leaf mass (g) | Dry bulb mass (g) | Yield (t·ha$^{-1}$) |
|---|---|---|---|---|---|---|---|
| N-source: Ammonium sulphate | | | | | | | |
| 0 | 21.2c | 24.8f | 170.4bc | 9.3b | 3.9c | 8.8bcd | 15.5b |
| 50 | 22.4bc | 27.7e | 190.7b | 10.5b | 4.6b | 10.2c | 15.9a |
| 100 | 23.5bc | 30.7d | 200.7b | 12.4a | 5.5ab | 12.4bc | 19.2a |
| 150 | 24.5b | 33.8c | 269.4a | 13.9a | 6.3a | 13.8b | 20.6a |
| 200 | 26.5a | 38.3b | 331.8a | 14.4a | 7. 5a | 18.0a | 23.6a |
| N-source: Calcium nitrate | | | | | | | |
| 0 | 21.2c | 24.80f | 170.4bc | 9.3b | 3.9c | 8.8cd | 15.5b |
| 50 | 22.9bc | 28.66e | 208.4b | 11.4b | 5.4ab | 12.0bc | 16.5a |
| 100 | 23.9bc | 31.60d | 252.5b | 13.5a | 5.8ab | 13.3b | 19.6a |
| 150 | 25.2b | 37.40b | 287.1a | 14.4a | 6.8a | 15.6b | 21.2a |
| 200 | 26.5a | 45.90a | 365.6a | 16.3a | 7.4a | 19.9a | 23.5a |
| Response | L** | L** | L** | L** | L** | L** | L** |

Means followed by the same letter within a column are not significantly different at p≤ 0.05 level of probability

Table 4.3 Effect of ammonium sulphate during the 2006/2007 growing season on *T. violacea* leaf tissue N

| | Sampling dates and N % | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Nitrogen ($kg \cdot ha^{-1}$) | July | August | September | October | November | December | January | February | March | April |
| 0 | 0.95b | 1.19b | 0.64d | 1.27b | 1.13c | 0.88b | 0.94b | 0.71c | 0.73b | 0.81d |
| 50 | 1.83a | 2.21a | 2.26c | 2.15a | 1.46bc | 1.49a | 1.62a | 2.31b | 2.49a | 1.73c |
| 100 | 1.90a | 2.27a | 2.49bc | 2.18a | 1.73ab | 1.57a | 1.69a | 2.35ab | 2.57a | 1.83c |
| 150 | 1.91a | 2.39a | 2.76ab | 2.23a | 1.74ab | 1.75a | 1.89a | 2.39ab | 2.62a | 2.25b |
| 200 | 1.99a | 2.43a | 2.80a | 2.34a | 1.91a | 1.86a | 2.07a | 2.52a | 2.82a | 2.81a |
| Response | L** | L** | L** | L** | L** | L** | L** | L** | L** | L** |

Means followed by the same letter within a column are not significantly different at $p \leq 0.5$ level of probability

Table 4.4 Effect of calcium nitrate during the 2006/2007 growing season on *T. violacea* leaf tissue N

| | Sampling dates and N % | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Nitrogen ($kg \cdot ha^{-1}$) | July | August | September | October | November | December | January | February | March | April |
| 0 | 0.95c | 0.92c | 0.64c | 0.86b | 1.13c | 0.88c | 0.94b | 0.71b | 0.71b | 0.81c |
| 50 | 2.03b | 2.24b | 2.46b | 1.16a | 1.71b | 1.57b | 1.69a | 2.25a | 2.57a | 1.83b |
| 100 | 2.04b | 2.35b | 2.64ab | 1.22a | 1.73b | 1.66ab | 1.70a | 2.31a | 2.57a | 2.10b |
| 150 | 2.16ab | 2.39ab | 2.77a | 1.29a | 1.79ab | 1.76ab | 1.79a | 2.31a | 2.70a | 2.63a |
| 200 | 2.38a | 2.63a | 2.85a | 1.38a | 2.04a | 1.95a | 1.97a | 2.50a | 2.79a | 2.88a |
| Response | L** | L** | L** | L** | L** | L** | L** | L** | L** | L** |

Means followed by the same letter within a column are not significantly different at $p \leq 0.5$ level of probability

### 4.3.3 Effect of nitrogen on number of leaves

In most plants, leaf senescence occurs because of the natural biological process of ageing, but it can also be stimulated by nutrient deficiency. Rapid premature leaf death normally occurs in unfertilized plants during the flowering period (Thomas & Staddart, 1980). The effect of nitrogen fertilization (ammonium sulphate and calcium nitrate) had a significant effect on the number of leaves per plant (Table 4.2). The highest number of leaves was found at 200 $kg \cdot ha^{-1}$ applications. Therefore, nitrogen application increased the number of leaves produced. Highest number of leaves produced at 200 $kg \cdot ha^{-1}$ N and the highest percentage of leaf tissue N were 1.97, 2.50, 2.79 and 2.88% which was found from plants that was treated with calcium nitrate in January, February, March and April, respectively (Table 4.4). The same trend apply to the plants treated with ammonium sulphate (Table 4.3) as *T. violacea* plants treated with calcium nitrate resulted in significantly more number of leaves as compared to plants treated with ammonium sulphate.

### 4.3.4 Effect of nitrogen on leaf area

Plants that were treated with calcium nitrate produced a greater leaf area as compared to plants treated with ammonium sulphate (Table 4.2). The results are in agreement with findings of Elia, Santamaria & Serio (1998). There were no significant differences between 150 and 200 $kg \cdot ha^{-1}$ for both N-sources, but significant differences were obtained between unfertilized and fertilized plants. The percentage leaf tissue N was the lowest at 0.64% in untreated plants and 2.85% with the ammonium sulphate treatment when plants were harvested in September (Table 4.7).

### 4.3.5 Effect of nitrogen on number of new tillers

During the first three sampling dates (July, August and September 2006) *T. violacea* plants did not develop any new tillers. Therefore during these months

the development of new tillers was not influenced by nitrogen nutrition. As from October 2006 plants started to develop new tillers. Number of new tillers increased significantly with an increase in N applied, regardless of the source of N applied to the plants (Table 4.2).

#### 4.3.6 Effect of nitrogen on dry leaf and dry bulb mass

Nitrogen application increased dry leaf and bulb mass of *T. violacea* plants (Table 4.2). Neither leaf nor bulb dry mass of *T. violacea* was significantly influenced by different nitrogen sources. The application of 200 $kg \cdot ha^{-1}$ produced the greatest dry leaf and bulb mass. The dry bulb mass increased with the increase of nitrogen applied and the bulb tissue N found with April harvest was 0.73% for untreated and 1.30% and 1.36% calcium nitrate and ammonium sulphate respectively.

#### 4.3.7 Effect of nitrogen on total plant yield

Nitrogen fertilization had a significant effect on plant yield (Table 4.2). At the end of the growing season, significant differences in harvested yields were obtained among the different treatments applied. The highest yields were obtained at 200 $kg \cdot ha^{-1}$ regardless of nitrogen source supplied to the plants. Therefore, the higher the nitrogen applied to the plant, the higher the yield obtained. The average yield ranged from 15.5 $t \cdot ha^{-1}$ for untreated plants to 23.6 $t \cdot ha^{-1}$ for ammonium sulphate treated plants and 23.5 $t.ha^{-1}$ for calcium nitrate treated plants.

#### 4.3.8 Effect of nitrogen on flowering

Fismes, Vong, Guckert & Frossard (2000) reported that sulphur is essential for plants during the vegetative and the reproductive growth stages. Flowering period of *T. violacea* plants lasted throughout the summer months (from October to March 2006/7). There were significant results between ammonium sulphate

and calcium nitrate on number of flower produced (Figure 4.1). Number of flowers increased with each level of ammonium sulphate applied up to 150 kg·ha$^{-1}$ N, but then decreased at 200 kg·ha$^{-1}$ N. However, number of flowers was greatest with 200 kg·ha$^{-1}$ N for plants treated with calcium nitrate. Furthermore, significantly more flowers were obtained with calcium nitrate than with ammonium sulphate. Due to higher number of new tillers produced with calcium nitrate (Table 4.2).

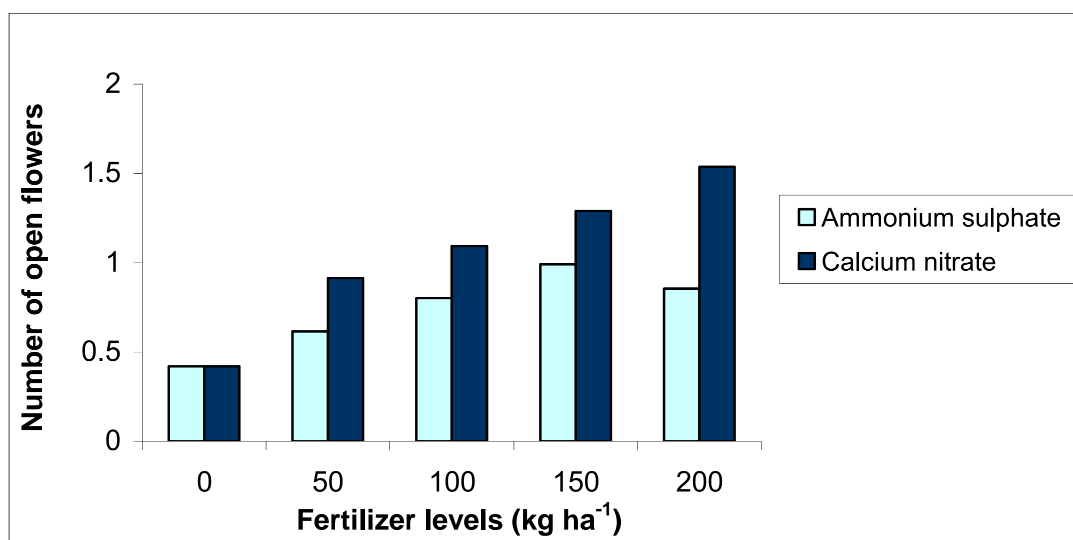


Figure 4.1 Effects of fertilizer on number of open flowers produced with each fertilizer application on *T. violacea*

#### 4.3.9 Plant nutrient analysis

Fertilizer Society of South Africa (FSSA) (2003) stated that plant nutrient analysis is done to determine the nutrient status of the plant which is used together with soil analysis results and it functions as a useful guideline determining nutrient deficiencies and for fertilizer recommendation in that specific plant.

### 4.3.10 Nitrogen concentration in *T. violacea* leaves and bulbs

Nitrogen concentration in *T. violacea* plants followed the same trend regardless of N source (Tables 4.3 and 4.4). In general, most of the significant differences in tissue N concentration were between untreated and treated plants. Also, less N accumulated in the leaves during summer (flowering period) compared to other season of the year. Plant height, leaf number, leaf area and dry leaf mass increased with each level of N application (Table 4.2). Leaf tissue N concentration increased with each level of N application at the last sampling date (April 2007). The highest leaf tissue N percentage was obtained from nitrogen application of 200 $kg \cdot ha^{-1}$ where calcium nitrate was 2.88% and the lowest was 0.81% for untreated plants. Similar results were found with ammonium sulphate applied to plants.

*T. violacea* plants accumulate less nitrogen in the bulbs than in the leaves (Tables 4.5 & 4.6). Also, these appear to be less the cause of seasonal effect on bulb N accumulation than on leaf N accumulation.

Table 4.5 Effect of ammonium sulphate during the 2006/2007 growing season on *T. violacea* bulb tissue N

| | Sampling dates and N % | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Nitrogen (kg·ha$^{-1}$) | July | August | September | October | November | December | January | February | March | April |
| 0 | 0.89a | 0.83c | 0.64a | 1.18a | 1.07a | 0.94a | 0.85a | 0.75a | 0.81a | 0.73c |
| 50 | 0.98a | 0.93b | 0.71a | 1.22a | 1.15a | 1.02a | 0.98a | 0.80a | 0.84a | 0.93bc |
| 100 | 1.02a | 1.13b | 0.76a | 1.29a | 1.19a | 1.07a | 0.98a | 0.84a | 0.84a | 1.06b |
| 150 | 1.03a | 1.14a | 0.87a | 1.34a | 1.25a | 1.18a | 1.06a | 0.92a | 0.89a | 1.31a |
| 200 | 1.16a | 1.20a | 0.92a | 1.43a | 1.33a | 1.29a | 1.21a | 0.98a | 0.99a | 1.36a |
| Response | L** | L** | L** | L** | L** | L** | L** | L** | L** | L** |

Means followed by the same letter within a column are not significantly different at $p \leq 0.5$ level of probability

Table 4.6 Effect of calcium nitrate during the 2006/2007 growing season on *T. violacea* bulb tissue N

| | Sampling dates and N % | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Nitrogen ($kg \cdot ha^{-1}$) | July | August | September | October | November | December | January | February | March | April |
| 0 | 0.89b | 0.83c | 0.64b | 1.18a | 1.07b | 0.94a | 0.98a | 0.75a | 0.81a | 0.73c |
| 50 | 1.07a | 1.18b | 0.76a | 1.25a | 1.16b | 0.99a | 1.06a | 0.78a | 0.85a | 0.93bc |
| 100 | 1.17a | 1.23a | 0.80a | 1.29a | 1.25ab | 1.07a | 1.13a | 0.80a | 0.87a | 1.14b |
| 150 | 1.22a | 1.23a | 0.88a | 1.35a | 1.29ab | 1.15a | 1.18a | 0.83a | 0.91a | 1.22a |
| 200 | 1.29a | 1.33a | 0.94a | 1.41a | 1.45a | 1.25a | 1.25a | 0.87a | 0.94a | 1.30a |
| Response | L** | L** | L** | L** | L** | L** | L** | L** | L** | L** |

Means followed by the same letter within a column are not significantly different at $p \leq 0.5$ level of probability

### 4.3.11 Sulphur concentration in *T. violacea* leaves and bulbs

Application of ammonium sulphate is used mainly to improve *T. violacea* plant growth and quality, and it has been noted that sulphate improves both plant growth and reproductive tissues (flower and seed pods) (Fismes *et al.*, 2000). According to the plant analysis results, leaf nitrogen was always greater than leaf sulphur. This is in agreement with findings of Beaton, Fox & Tabatabai (1986) who indicated that many crops take up a small amount of sulphur content when compared to other elements. Increasing ammonium sulphate applied from 0 to 200 $kg \cdot ha^{-1}$ increased leaf tissue S from 0.99% to 1.58% for plants sampled in April 2007. Surprisingly, ammonium sulphate application did not have a major influence in S accumulation in *T. violacea* leaves (Table 4.7). Most of the influence occurred at the last sampling date (April 2007). The S accumulation in *T. violacea* plants is important because it is linked with the improvement of plant bioactivity (Nteso & Pretorius, 2006).

In general, ammonium sulphate application had little influence on S accumulation in *T. violacea* bulbs (Table 4.8). But even where no significances were obtained, there was a trend for higher values of S in the bulbs with each increase in ammonium sulphate application.

Table 4.7 Effect of ammonium sulphate during the 2006/2007 growing season on *T. violacea* leaf tissue S

| | Sampling dates and S % | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Nitrogen ($kg \cdot ha^{-1}$) | July | August | September | October | November | December | January | February | March | April |
| 0 | 0.69a | 1.06a | 0.79b | 0.81b | 0.79a | 0.48c | 0.77a | 0.88a | 1.11a | 0.99c |
| 50 | 0.77a | 1.06a | 1.09a | 0.91a | 0.86a | 0.61b | 0.89a | 0.89a | 1.28a | 1.20b |
| 100 | 0.78a | 1.12a | 1.12a | 0.92a | 0.89a | 0.83a | 0.92a | 0.95a | 1.34a | 1.38ab |
| 150 | 0.79a | 1.17a | 1.183a | 0.96a | 0.91a | 0.85a | 0.93a | 0.97a | 1.35a | 1.45ab |
| 200 | 0.87a | 1.21a | 1.26a | 0.99a | 0.94a | 0.88a | 0.97a | 0.98a | 1.44a | 1.58a |
| Response | L** | L** | L** | L** | L** | L** | L** | L* | L** | L** |

Means followed by the same letter within a column are not significantly different at $p \leq 0.5$ level of probability

Table 4.8 Effect of ammonium sulphate during the 2006/2007 growing season on *T. violacea* bulb tissue S

| | Sampling dates and S % | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Nitrogen ($kg{\cdot}ha^{-1}$) | July | August | September | October | November | December | January | February | March | April |
| 0 | 0.72a | 0.67b | 0.62b | 0.77a | 0.80a | 0.73c | 0.81a | 0.89a | 1.13a | 1.15a |
| 50 | 0.75a | 1.06a | 1.10a | 0.88a | 0.85a | 0.76bc | 0.92a | 0.91a | 1.25a | 1.23a |
| 100 | 0.79a | 1.16a | 1.11a | 0.91a | 0.87a | 0.81ab | 0.93a | 0.96a | 1.32a | 1.29a |
| 150 | 0.84a | 1.18a | 1.18a | 0.93a | 0.89a | 0.85a | 0.94a | 0.97a | 1.35a | 1.38a |
| 200 | 0.92a | 1.22a | 1.21a | 0.98a | 0.92a | 0.89a | 0.98a | 1.01a | 1.33a | 1.48a |
| Response | L** | L** | L** | L** | L** | L** | L** | L** | Q** | L** |

Means followed by the same letter within a column are not significantly different at $p \leq 0.5$ level of probability

### 4.3.12 Calcium concentration in *T. violacea* leaves and bulbs

Calcium is an important constituent of plant tissue and has a vital role in maintaining and modulating various cell functions and reduced onion bulbs flaking and increase bulb yield (Ghoname, El-Bassiony, Riadand & El-Baky, 2007). In Table 4.9 and 4.10 there were no significances obtained. There was a trend for higher values of a Ca in the leaves (Table 4.9) with each increase in calcium nitrate application. The application of calcium and potassium were recommended to prevent pre and post harvest diseases and to increase post harvest yield and shelf life quality. The application of calcium on onion and garlic was related to increase pungency and bioactivity percentage (Ghoname *et al*., 2007).

Table 4.9 Effect of calcium nitrate during the 2006/2007 growing season on *T. violacea* leaf tissue Ca

| | Sampling dates and Ca % | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Nitrogen ($kg \cdot ha^{-1}$) | July | August | September | October | November | December | January | February | March | April |
| 0 | 0.92 | 0.86 | 0.91 | 0.97 | 0.63 | 0.72 | 0.91 | 0.91 | 0.75 | 0.63 |
| 50 | 1.45 | 1.46 | 1.72 | 1.99 | 1.76 | 1.42 | 1.32 | 1.63 | 1.67 | 1.58 |
| 100 | 1.84 | 1.98 | 2.33 | 2.43 | 1.78 | 1.68 | 1.48 | 1.97 | 1.95 | 2.32 |
| 150 | 2.11 | 2.23 | 2.69 | 2.84 | 1.82 | 1.87 | 1.67 | 2.34 | 2.55 | 2.65 |
| 200 | 2.21 | 2.43 | 2.88 | 2.92 | 1.93 | 1.99 | 1.86 | 2.67 | 2.78 | 2.87 |
| Response | L** | L** | L** | L** | L** | L** | L** | L** | L** | L** |

Means followed by the same letter within a column are not significantly different at $p \leq 0.5$ level of probability

Table 4.10 Effect of calcium nitrate during the 2006/2007 growing season on *T. violacea* bulb tissue Ca

| | Sampling dates and Ca % | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Nitrogen ($kg \cdot ha^{-1}$) | July | August | September | October | November | December | January | February | March | April |
| 0 | 0.08 | 0.06 | 0.06 | 0.08 | 0.92 | 0.87 | 0.77 | 0.08 | 0.08 | 0.07 |
| 50 | 0.94 | 1.34 | 0.45 | 1.41 | 1.53 | 1.22 | 1.23 | 0.93 | 0.98 | 1.15 |
| 100 | 1.23 | 1.58 | 0.63 | 1.59 | 1.79 | 1.48 | 1.34 | 1.04 | 1.06 | 1.36 |
| 150 | 1.35 | 1.67 | 0.88 | 1.68 | 1.97 | 1.73 | 1.66 | 1.09 | 1.17 | 1.55 |
| 200 | 1.81 | 1.89 | 1.11 | 1.91 | 2.11 | 1.95 | 1.86 | 1.19 | 1.26 | 1.86 |
| Response | L** | L** | L** | L** | L** | L** | L** | L** | L** | L** |

Means followed by the same letter within a column are not significantly different at p≤ 0.5 level of probability

## 4.4 CONCLUSIONS

The findings of this study convincingly showed that ammonium sulphate and calcium nitrate affect plant growth and yield of *T. violacea* plants. Untreated plants struggled to grow due to a lack of nitrogen nutrition and resulted in poor plant growth and low yield. The increase in nitrogen nutrition (ammonium sulphate and calcium nitrate) resulted in an increase in plant height, leaf area, new tillers, leaf and bulb dry mass as well as yield.

According to the results of this study, more nitrogen accumulated mainly in plant leaves than in bulbs and similarly with sulphate accumulation. The highest leaf tissue N percentage was obtained from nitrogen application of 200 $kg \cdot ha^{-1}$ where calcium nitrate was 2.88% and the lowest was 0.81% in untreated plants. Similar trend occurs to ammonium nitrate applied plants. Greater leaf tissue S accumulation was greatest at 200 $kg \cdot ha^{-1}$ at 1.58% and the lowest was 0.99% for untreated plants. Similar trend occurs to bulb tissue S accumulation for plants treated with ammonium sulphate. Therefore, results from the study have potential of being used as a reference in developing a nutrition recommendation guide, planned for optimal *T. violacea* performance.

## 4.5 SUMMARY

The study was undertaken to determine the effect of two nitrogen sources (ammonium sulphate and calcium nitrate) at 0, 50, 100, 150 and 200 $kg \cdot ha^{-1}$ on growth and yield of *Tulbaghia violacea*. The experiment was conducted in a field at the Experimental Farm of the Agricultural Research Council, Roodeplaat.

Plant growth was determined by measuring plant height, leaf area, leaf number, number of new tillers, dry leaf mass, dry bulb mass and yield. The parameters were collected monthly from July 2006 until April 2007 (ten months). Fresh leaves and bulbs were placed in an oven for two weeks at 40ºC so that the plant

materials could dry out. At each sampling date, plant materials were sent to the University of Pretoria for chemical analysis.

*T. violacea* plant height increased together with an increase in nitrogen applied. Maximum plant heights were obtained at 200 $kg{\cdot}ha^{-1}$ regardless of nitrogen source (ammonium sulphate and calcium nitrate). Number of leaves was highest when plants were produced at 200 $kg{\cdot}ha^{-1}$ and treated with calcium nitrate. Leaf area increased with an increase in nitrogen fertilizer supplied to the plants. However, plants supplied with calcium nitrate had higher leaf areas as compared to ammonium sulphate fed plants.

The number of tillers, dry leaf and bulb mass and yield were significantly affected by nitrogen nutrition. N and S accumulation in plant leaves increased as the levels of ammonium sulphate applied increased and the best yield was obtained at the highest N level. S that was found in the plant is important, since it will improve the bioactivity of the plant. S accumulation in the plant was greater at the end of the growing season in summer compared to other seasons of the year. Yield obtained from both N sources were significantly different between the unfertilized and fertilized plants. Yields of both N sources were not significantly different.

# CHAPTER 5

# ANTIFUNGAL PROPERTIES OF *TULBAGHIA VIOLACEA* HARV. (WILD GARLIC) PLANT EXTRACTS AGAINST *ALTENARIA SOLANI* AND *SCLEROTIUM ROLFSII*

## 5.1 INTRODUCTION

*Tulbaghia violacea* is an important indigenous herb in South Africa that belongs to the family Alliacea (Van Wyk *et al*., 1997). This plant has medicinal properties and has been widely used by South African traditional healers for the treatment of flu, fever, cold, tuberculosis, cancer of the oesophagus and asthma ( Van Wyk *et al*., 1997; Kubec *et al*., 2002). It is used in South African communities due to its anti-fungal, antibacterial and antiviral properties. However, Kubec *et al*. (2002) found that extensive consumption of this plant has a negative effect which leads to abdominal pain, inflammation of the intestine and gastroenteritis.

Early blight disease, which is caused by *Alternaria solani*, is a major disease that limits the economic production of potato and tomato in South Africa (Blachinski, Shtienberg, Dinoor, Kafkafi, Sujkowski, Zitter & Fry, 1996). This fungus causes infections on leaf, stem and fruit of plants and can result in harsh damage during all stages of plant development (Floolad & Ntahimpera, 2000).

*Sclerotium rolfsii* is a soilborne fungal pathogen that causes common southern stem rot or southern blight on a wide range of agricultural and horticultural crops, weeds and forest trees (Flores-Moctezuma, Montes-Belmont, Jimenez-Perez, & Nava-Juarez, 2005). These diseases are widely distributed throughout the world including South Africa. The pathogen causes plant rot, which reduces yield dramatically (Cilliers, Pretorius & Van Wyk, 2003).

*A. solani* can be prevented by the use of fungicides, including azoxystrobin, potassium bicarbonate, hydrogen dioxide, maneb and mancozeb (Floolad & Ntahimpera, 2000). The fungicides control of *S. rolfsii* includes Methyl Bromide and Methyl Bromide/Chloropicrin (Cilliers *et al*., 2003). Both diseases can be controlled culturally by means of crop rotation as a cultural control with resistant *Allium* species. Therefore, the use of two different type of fertilizer to improve yield and quality is important to this crop. High yield of *T. violacea* with better bulb extract is required to fight plant pathogens *A. solani* and *S. rolfsii* as an organic farming.

The aim of this study was to determine the ideal N: ammonium sulphate and calcium nitrate (0, 50, 100, 150 and 200 $kg \cdot ha^{-1}$) levels for optimal bioactivity in *T. violacea* plant. To determine the antifungal effects of *T. violacea* bulb extracts against plant pathogens *Altenaria solani* and *Sclerotium rolfsii*.

## 5.2 MATERIALS AND METHODS

The plant materials of *T. violacea* that were collected from the second field experiment (Chapter 4) were used for screening in a laboratory. Plant materials were screened against *S. rolfsii* and *A. solani* plant pathogens (Chapter 3 materials and methods).

## 5.3 RESULTS AND DISCUSSION

### 5.3.1 Effects of ammonium sulphate on *T. violacea* bulb extracts

Ammonium sulphate applied at 0, 50, 100, 150 and 200 $kg \cdot ha^{-1}$ had an effect on bioactivity. The application of ammonium sulphate increased the percentage bioactivity of *T. violacea* bulb extracts against *S. rolfsii* and *A. solani* as compared to the control (Figure 5.1). The highest bioactivity was obtained at 100 $kg \cdot ha^{-1}$ ammonium sulphate fertilizer as an optimum level. Thereafter, bioactivity decreased at 150 and 200 $kg \cdot ha^{-1}$ ammonium sulphate.

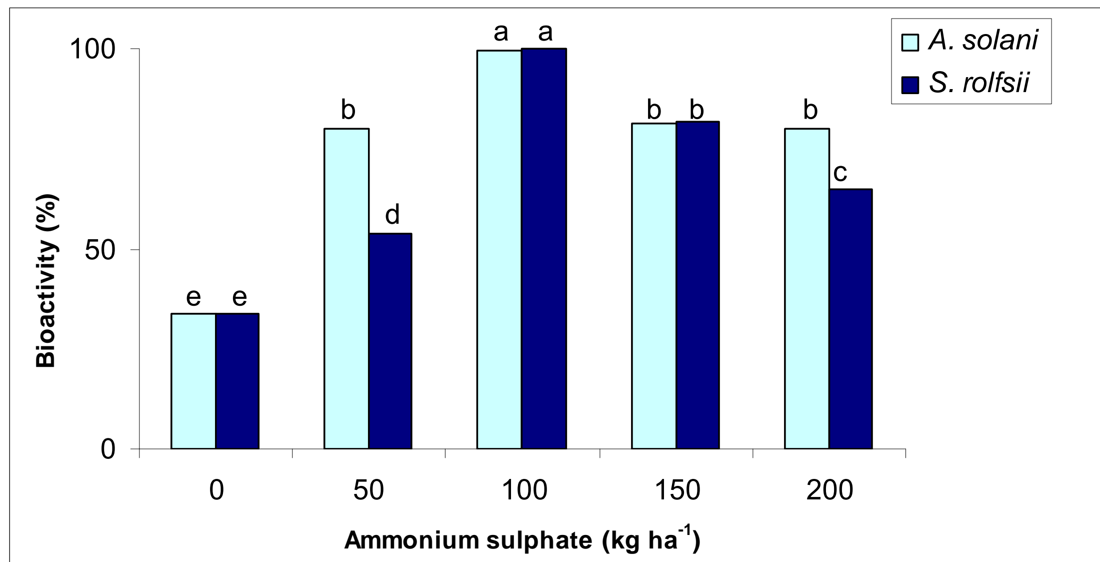


Figure 5.1 Effects of ammonium sulphate on the bioactivity of *T. violacea* bulb extracts against *S. rolfsii* and *A. solani*

**5.3.2 Effects of calcium nitrate on *T. violacea* bulb extracts**

The application of calcium nitrate at 0, 50, 100, 150 and 200 kg·ha$^{-1}$ significantly decreased the bioactivity of the extracts against both fungi. The more the calcium nitrate applied, the poorer was the bioactivity exhibited by the bulb extracts. Therefore, the application of calcium nitrate resulted in poor bioactivity when compared with the untreated plants (Figure 5.2).

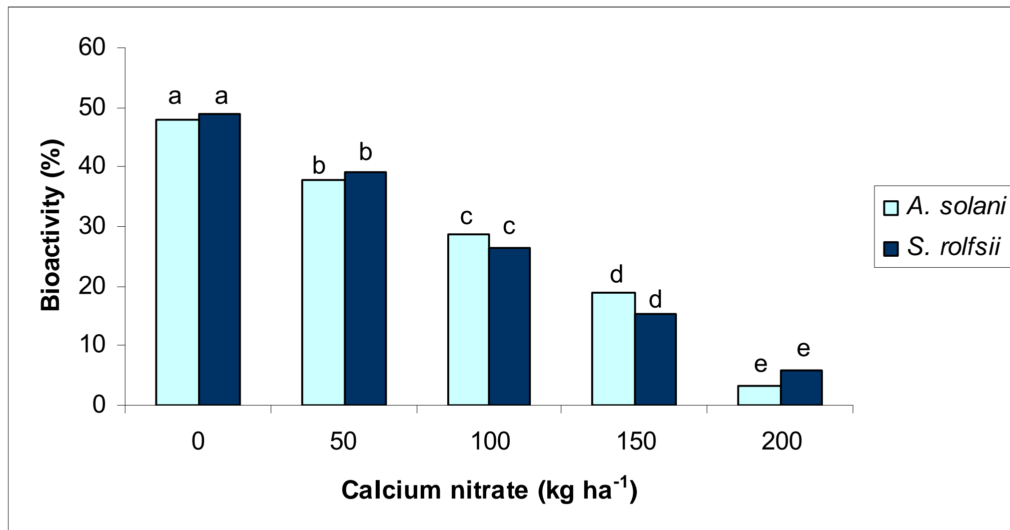


Figure 5.2 Effects of calcium nitrate on the bioactivity of *T. violacea* bulb extracts against *S. rolfsii* and *A. solani*

The decrease of the bioactivity could either be due to low bioactivity or dilution of biological activity or another cyclic condition or blockage of bioactivity that had been influenced by calcium nitrate to the plants. Results obtained from the plants treated with calcium nitrate indicated that calcium nitrate improves plant yield but with very low bioactivity. The application of calcium nitrate can therefore be recommended in the production of *T. violacea*, which are planted for horticultural purposes and not for medicinal use.

### 5.3.3 Effects of harvesting dates on *T. violacea* bulb extracts

The experiment was planted during autumn season in April 2006. Harvesting started in July 2006 until April 2007. During the first sampling month (July 2006) of *T. violacea* plants produced lower of bioactivity. The highest bioactivity was obtained in April 2007 (Figure 5.3). The lowest bioactivity was obtained during the months of November and December 2006. The plant is then in a reproductive stage, uses its energy for flowering. The decrease in bioactivity could be associated with a cyclic pattern which can be attributed to (specific growth parameters such as) bulb initiations, plus an increase in minimum and maximum

temperatures during these months. In January 2007, bioactivity started to improve and continued increasing.

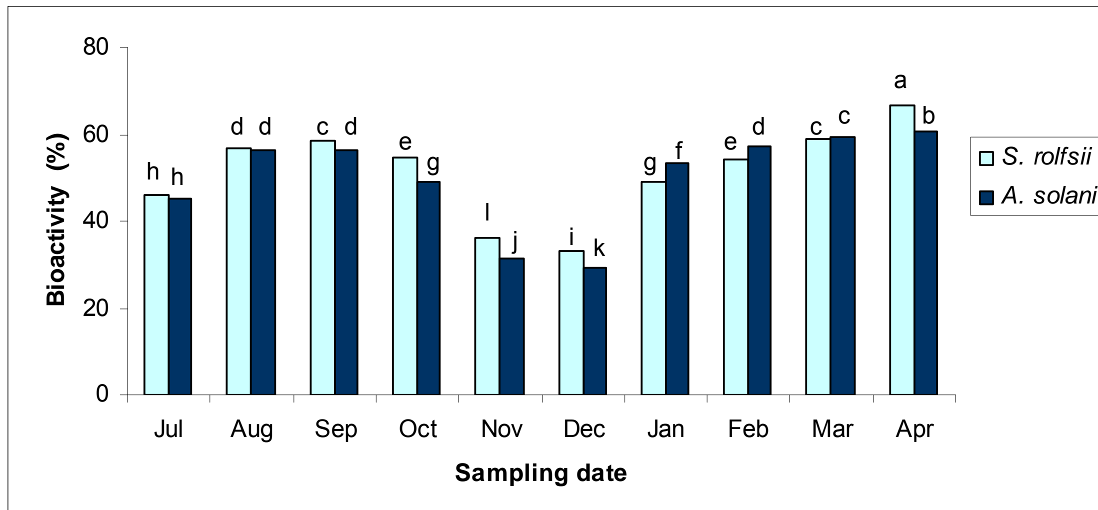


Figure 5.3 Effects of harvesting date and ammonium sulphate on bioactivity of bulb extracts against *S. rolfsii* and *A. solani*

### 5.3.4 Interactions between ammonium sulphate and calcium nitrate

Figure 5.4 shows the interactions between the two nitrogen fertilizers (ammonium sulphate and calcium nitrate) on *T. violacea* extracts. Ammonium sulphate resulted in higher bioactivity when compared to calcium nitrate fed plants for both plant pathogens. Plants that were treated with calcium nitrate (50 to 200 $kg{\cdot}ha^{-1}$) had significantly lower bioactivity as compared to the control. Conversely, plants that were treated with ammonium sulphate from 50 to 200 $kg{\cdot}ha^{-1}$ resulted in significantly improved bioactivity.

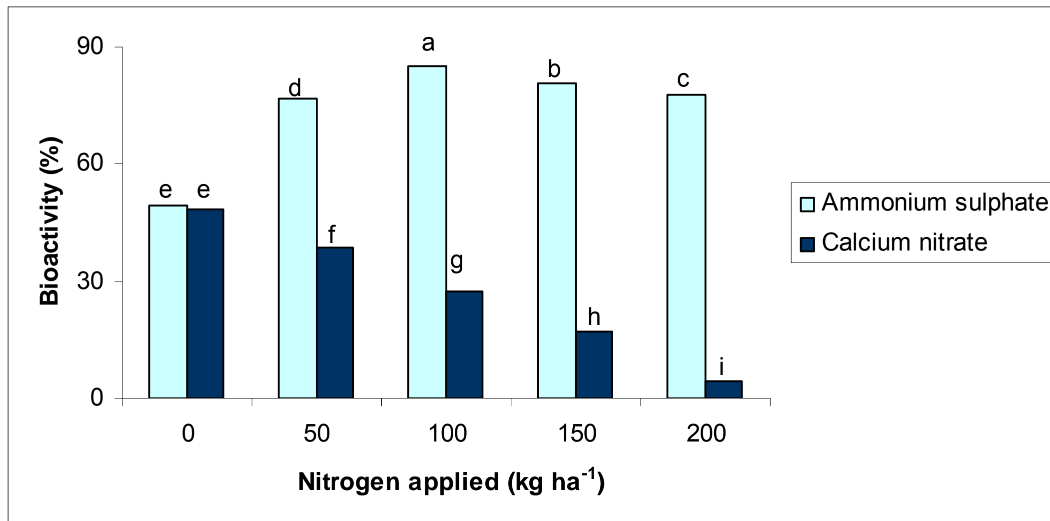


Figure 5.4 Effects of ammonium sulphate and calcium nitrate on the bioactivity of *T. violacea* bulb extracts against *S. rolfsii* and *A. solani*

## 5.4 CONCLUSIONS

Application of ammonium sulphate fertilizer increased the percentage bioactivity of *T. violacea* bulb extracts against *S. rolfsii* and *A. solani* as compared to untreated plants. The highest bioactivity was obtained at 100 $kg \cdot ha^{-1}$ ammonium sulphate fertilizer. Plants that were treated with calcium nitrate significantly decreased the bioactivity of the extracts against both fungi. *T. violacea* produced the lowest bioactivity during November and December 2006 and the highest bioactivity was obtained in April 2007. In general, better bioactivity was produced during winter than in summer months. Therefore, *T. violacea* that has been treated with ammonium sulphate fertilizer is recommended to be harvested during winter months than in a hot summer months when plants are actively producing flowers.

## 5.5 SUMMARY

An experiment was conducted in the field at the Experimental Farm of the Agricultural Research Council - Vegetable and Ornamental Plant Institute (ARC-

VOPI) at Roodeplaat, Pretoria. The study was undertaken to determine the influence of ammonium sulphate and calcium nitrate nutrition on the bioactivity of *T. violacea* bulb extracts. It was achieved by screening extracts of plants that were harvested monthly for ten months and screened against plant pathogens *Alternaria solani* and *Sclerotium rolfsii*.

In this study the crude extracts from *T. violacea* bulb extracts of plants treated with ammonium sulphate significantly inhibited the growth of plant pathogenic fungi, *S. rolfsii* and *A. solani*, whereas extracts from plants treated with calcium nitrate showed a decrease in bioactivity. *T. violacea* plants that received ammonium sulphate at 50, 100, 150 and 200 $kg \cdot ha^{-1}$ had significantly greater yield when compared to the untreated plants. Plants that had received ammonium sulphate at 100 $kg \cdot ha^{-1}$ produced the highest bioactivity. Thereafter, the trend reduced at 150 and 200 $kg \cdot ha^{-1}$ ammonium sulphate. While plants that received ammonium sulphate at 50, 100, 150 and 200 $kg \cdot ha^{-1}$ had significantly decreased the bioactivity against *A. solani* and *S. rolfsii.* Harvesting started in July until April 2007. The lowest bioactivity was obtained during November and December 2006. The recommended harvesting time is around April and onwards when the plant would produce the highest bioactivity.

# CHAPTER 6

# INFLUENCE OF SEASONAL PLANTING ON YIELD AND QUALITY OF WILD GARLIC (*TULBAGHIA VIOLACEA* HARV.)

## 6.1 INTRODUCTION

Wild garlic (*Tulbaghia violacea*) is a bulbous perennial plant, which is commonly known as isihaqa (Zulu), itswele lomlambo (Xhosa) and wilde knoffel (Afrikaans) (Van Wyk *et al*., 1997; Bungu, Frost, Brauns & Van de Venter, 2006). *T. violacea* is one of the plants which are a rich source of organo-sulphur compounds (Corzo-Martinez *et al*., 2007). It is an indigenous plant to South Africa but its leaves and bulbs are widely used as herbal remedy for various diseases all over the world (Harris, 2004).

Traditionally, *T. violacea* has been grown in Kwazulu Natal, Gauteng and Eastern Cape Province of South Africa in rocky grassland areas (Kubec *et al*., 2002). South African communities grew this crop for the treatment of fever, colds, asthma, tuberculosis and many more diseases (Van Wyk *et al*., 1997; Kubec *et al*., 2002; Bungu *et al*., 2006).

There is no information that exists about the optimal planting time for *T. violacea*. It is hypothesized that yield and quality obtained from this plant can differ as a result of different planting dates, seasons, and climate and soil conditions. In plants such as tulip and iris, Willits & Peet (1998) found that higher temperature interferes with their reproductive stages. Many plant species require specific temperatures at a specific growing stage.

The objective of this study was to determine the effects of planting season on the growth, yield and quality of bioactivity produced, with an aim of comparing autumn and winter planting seasons.

## 6.2 MATERIALS AND METHODS

The field experiment was conducted at the Experimental Farm of the Agricultural Research Council - Vegetable and Ornamental Plant Institute (ARC-VOPI) at Roodeplaat, Pretoria. The experiment was conducted from April 2006 to August 2007. A completely randomized block design consisting of nine treatments and four replications was used.

Planting materials of *T. violacea* bulbs were obtained from Lifestyle Seed Company based in the Free State Province. Treatments applied were as described in Chapter 4. Treatments were applied by means of top dressing at three month intervals (Table 4.1). Minimum and maximum temperatures and rainfall data were collected during the growing period (Tables 6.1 and 6.2).

Table 6.1 Monthly temperature and rainfall data for *T. violacea* plants sampled in autumn 2007

| Month | Ap | My | Jn | Jl | Au | Se | Oc | No | De | Ja | Fe | Mr | Ap | Mean |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Minimum Temperature (°C) | 7 | 0 | -3 | -2 | 0 | 7 | 13 | 15 | 17 | 15 | 15 | 14 | 11 | 8 |
| Maximum Temperature (°C) | 29 | 27 | 27 | 27 | 30 | 27 | 30 | 28 | 32 | 31 | 33 | 31 | 27 | 29 |
| Rainfall (mm) | 13 | 0 | 0 | 0 | 0 | 1.4 | 20 | 87 | 73 | 48 | 20 | 6.6 | 14 | 283 |

Table 6.2 Monthly temperature and rainfall data for *T. violacea* plants sampled in spring 2007

| Month | Au | Se | Oc | No | De | Ja | Fe | Mr | Ap | My | Jn | Jl | Au | Mean |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Minimum Temperature (°C) | 0 | 7 | 13 | 15 | 17 | 15 | 15 | 14 | 11 | 4 | 2 | 1.4 | 4 | 9 |
| Maximum Temperature (°C) | 30 | 27 | 30 | 28 | 32 | 31 | 33 | 31 | 27 | 24 | 21 | 21 | 24 | 28 |
| Rainfall (mm) | 0 | 1 | 20 | 87 | 73 | 48 | 20 | 6.6 | 14 | 0 | 32 | 9.6 | 0 | 311.2 |

Plants per plot were destructively harvested on a monthly basis from July 2006 to April 2007 for the autumn planting and November to August 2007 for the spring

planting season. Sampling data were collected for determination of number of leaves, plant height (cm), fresh and dry leaf mass (g), fresh and dry bulb mass (g), neck and bulb circumference (mm), leaf area ($cm^2$), number of flowers and yield.

Harvested plants were stored in an oven for two weeks at 40 $^{o}$C to dry-out. The dried samples were divided into two halves, with one-half for chemical analysis and the other half for screening of bioactivity.

Collected data were sent to the ARC - Institute of Agricultural Engineering (Biometry Unit) for statistical analysis using GenStat (2005). Differences between treatments were tested by means of analysis of variance (ANOVA). Treatment means were separated using LSD test at the 5 % level of significance.

## 6.3 RESULTS AND DISCUSSION

### 6.3.1 Weather conditions

Weather data on rainfall and temperature were collected from the experimental farm at ARC-VOPI. The average annual rainfall was about 283 mm occurring throughout autumn growing season (month to month). The average annual rainfall was about 311.2 mm occurring from spring growing season (August to August) (Tables 6.1 & 6.2).

### 6.3.2 Effect of seasonal planting on the growth of *T. violacea* plants

*T. violacea* plants were planted in two growing seasons (autumn and spring). Both experiments ran for 13 months. Data were collected monthly for ten months starting from the fourth month after transplanting.

Plant height of *T. violacea* plants fluctuated around 25 cm throughout the growing season. The highest plants were found during the third sampling month (September 2006) in the autumn experiment (Figure 6.1).

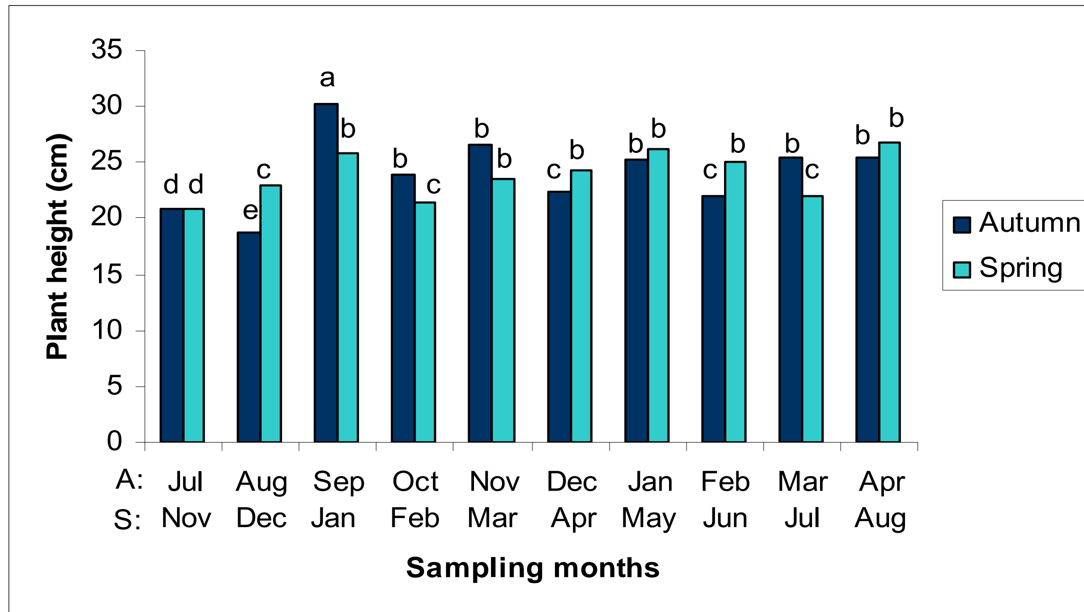


Figure 6.1 Effect of autumn and spring plantings on *T. violacea* plant height

For plants that were planted during spring season, leaf number increased up to the sixth sampling month (May 2007) (Figure 6.2). Thereafter, leaf number decreased due to very low night temperatures of -2 to -6ºC that were experienced for five days (data not shown). Number of leaves was adversely affected by very cold night temperatures during June month. After the cold spell, leaf number started to increase again from 22 to 48 leaves per plant. Autumn planting resulted in a higher leaf number of 56 leaves per plant than those of the spring planting season which obtained 49 leaves per plant.

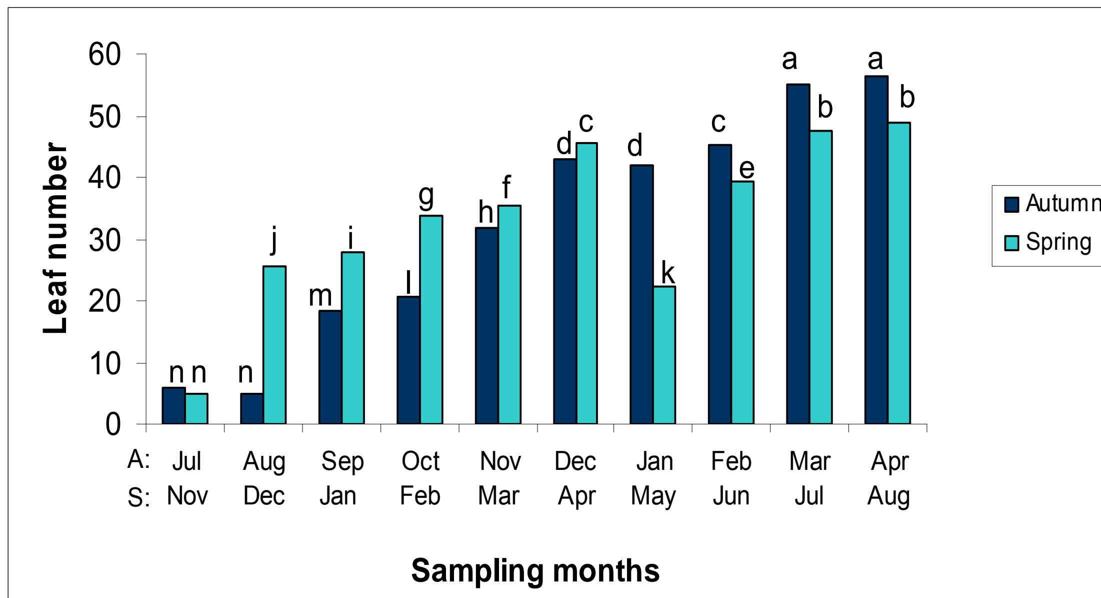


Figure 6.2 Effects of autumn and spring plantings on the number of leaves of *T. violacea*

Figure 6.3 indicates that *T. violacea* plants tolerated temperatures below -2°C to -6°C. *T. violacea* plants that were affected by low temperature recovered by producing new leaves. It was not all of the leaves that were affected. Some of the leaves showed tolerance to low temperatures (Figure 6.3). Also, it was not the whole experiment that was affected by the cold, but it was only a few plants that had suffered the cold spell.

Figure 6.3 Effects of low temperature on the aerial parts of *T. violacea* planted during spring

The results of leaf area obtained from the autumn planting were low for the first few sampling months, but increased dramatically after the seventh sampling month (January 2007). The results are in agreement with findings of Kulkarni *et al*. (2005) where *T. violacea* plants grown at a low temperature of 10ºC produced small plants compared to the ones planted at 25ºC. Winter planted plants produced low leaf areas at the first two sampling months but by the third sampling month, leaf area increased substantially (Figure 6.4).

The results of leaf area obtained on the seventh sampling month (May 2007) during the winter planting season indicated a decrease in leaf area due to the effect of very low temperatures but started to increase by the eighth sampling month (January 2007). Autumn planting results were in agreement with the findings of Keating, Evenson & Fukai (1982) where leaf area of cassava had an increase in plant growth.

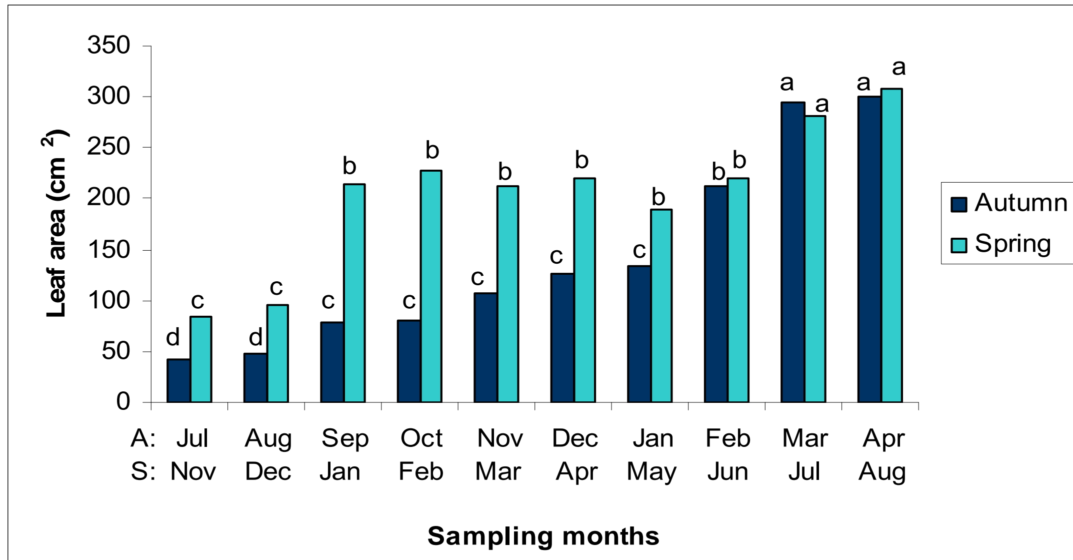


Figure 6.4 Effects of autumn and spring plantings on leaf area of *T. violacea*

Plants that were planted during autumn had not produced any new tillers by the first three sampling months. Autumn planting resulted in a lower number of new tillers of 12 tillers, when compared to winter planting of 16 new tillers per plant (Figure 6.5).

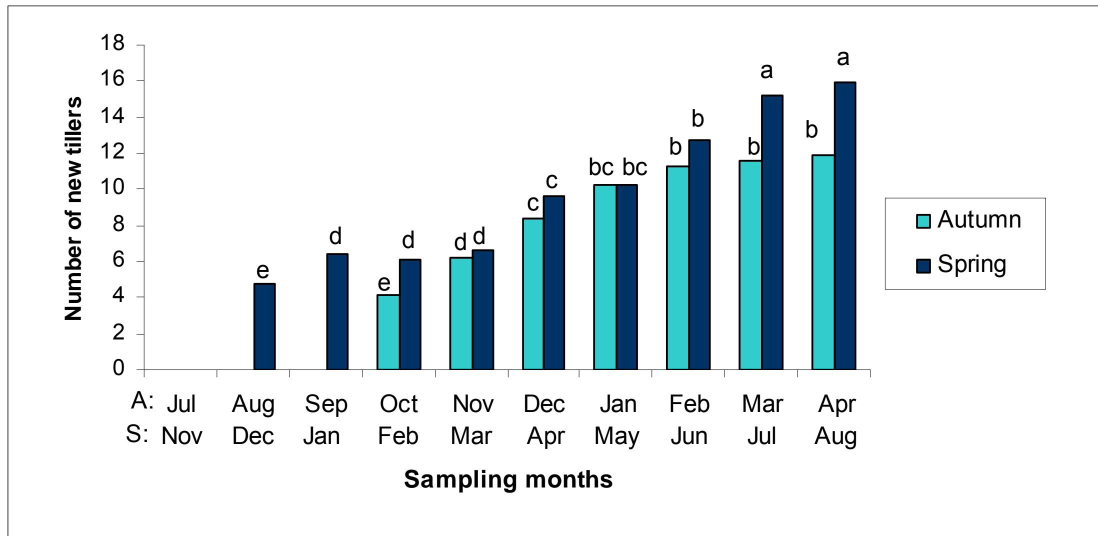


Figure 6.5 Effect of autumn and spring plantings on the number of new tillers of *T. violacea*

The results from the two planting seasons indicated that *T. violacea* plants started to flower in November 2006 to March 2007 (Figure 6.6) in both experiments. A higher number of flowers were found during the third sampling month (January 2007) in both experiments. However, plants from the autumn planting had a greater number of flowers at this sampling date, plants were physiologically older therefore more flowers were produced.

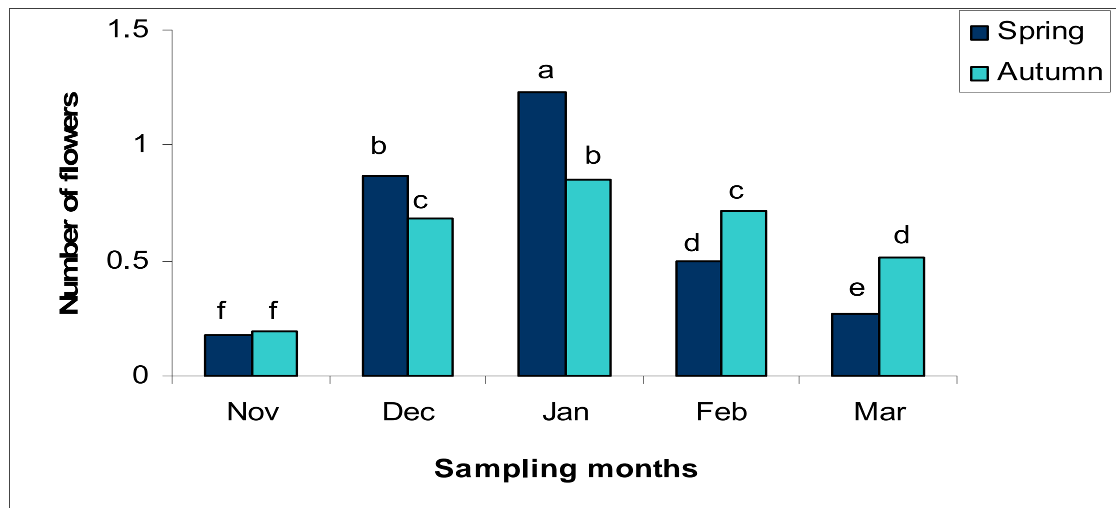


Figure 6.6 Effects of autumn and spring plantings on number of flowers of *T. violacea*

Plants from the autumn planting produced very low dry bulb mass at the first two sampling months (Figure 6.7). By the seventh sampling month (January 2007), however, autumn planting had plants with greater dry bulb mass than winter planting season. The growing temperatures were more favourable for autumn. Winter planting produced plants with greater dry bulb mass when compared with autumn planting during the first six sampling months.

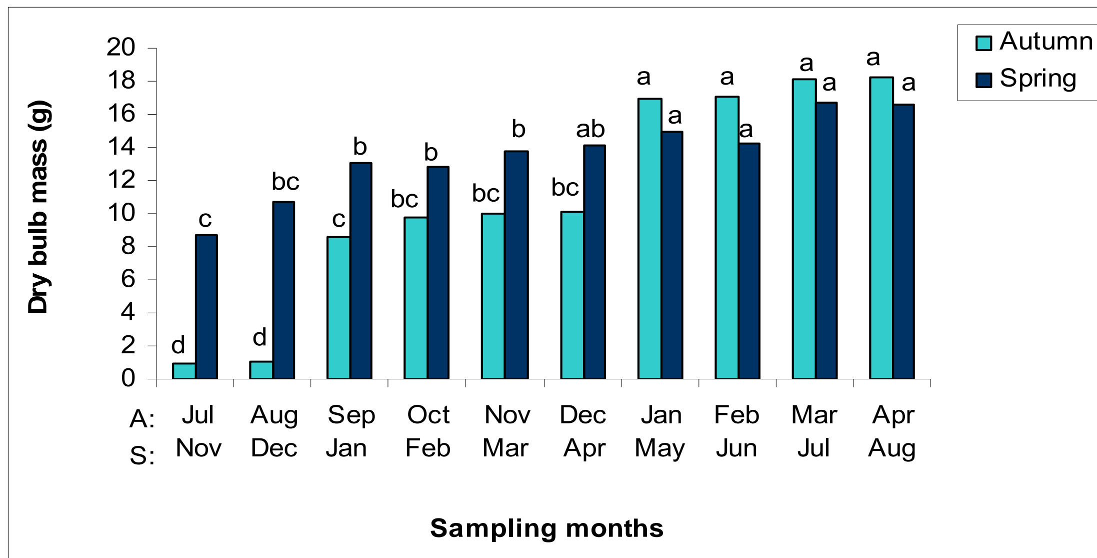


Figure 6.7 Effects of autumn and spring plantings on dry bulb mass of *T. violacea*

The results obtained from Figure 6.7 during winter planting season were in agreement with the findings of Kulkarni *et al*. (2005) that lower temperatures in winter stimulate plants to produce less shoot development and greater bulb formation than for a season with higher temperatures.

### 6.3.3 Effect of seasonal plantings on the total yield of *T. violacea*

The cumulative yield of *T. violacea* plants were computed at the end of each growing season. The first experiment (autumn) was planted in April 2006 and harvested in April 2007. The second experiment (spring) was planted in August 2006 and harvested in August 2007. Both experiments received the same treatments of fertilizers (5 levels of ammonium sulphate or 5 levels of calcium nitrate). The total yields obtained from both seasonal plantings are presented in Table 6.3.

Table 6.3 Effects of seasonal planting and nitrogen source on total yield of *T. violacea*

| | Ammonium sulphate | |
|---|---|---|
| N kg·ha$^{-1}$ | Autumn | Spring |
| 0 | 15.5b | 16.8c |
| 50 | 15.8ab | 17.4c |
| 100 | 19.2ab | 20.1bc |
| 150 | 20.6ab | 24.9ab |
| 200 | 23.6a | 27.4a |
| | Calcium nitrate | |
| 0 | 15.5b | 16.8c |
| 50 | 16.5ab | 17.7c |
| 100 | 19.6ab | 20.7bc |
| 150 | 21.0ab | 25.8ab |
| 200 | 23.5a | 30.8a |
| Significance | L** | L** |

Means followed by the same letter within a column are not significantly different at 5% level of probability

The findings from this study are in agreement with the results of Adamson & Coffelt (2005) that higher temperatures at the end of the autumn growing season results in reduced yield when compared with spring growing season. The results obtained from the two planting seasons indicated that total yield harvested from the winter planting was higher than for the autumn planting regardless of N source. Similar findings were reported by Van An, Frankow-Lindberg & Lindberg (2003) from two planting seasons, indicating that total yield from spring planting was higher than autumn planting. Kulkarni *et al*. (2005) found that *T. violacea* plants performed better at a temperature of up to 25$^{o}$C than at temperatures between 30 to 35$^{o}$C. Higher temperatures decreased production of *T. violacea* plants or resulted in poor plant growth.

### 6.3.4 Antifungal properties of *T. violacea* plants at two planting dates

Percentage bioactivity was determined from the two seasons, autumn and spring. Bioactivity of bulb extracts from the autumn planting season varied with sampling date (Figure 6.8). The highest bioactivity was in April 2007, while the lowest bioactivity was in the December 2006 sampling date.

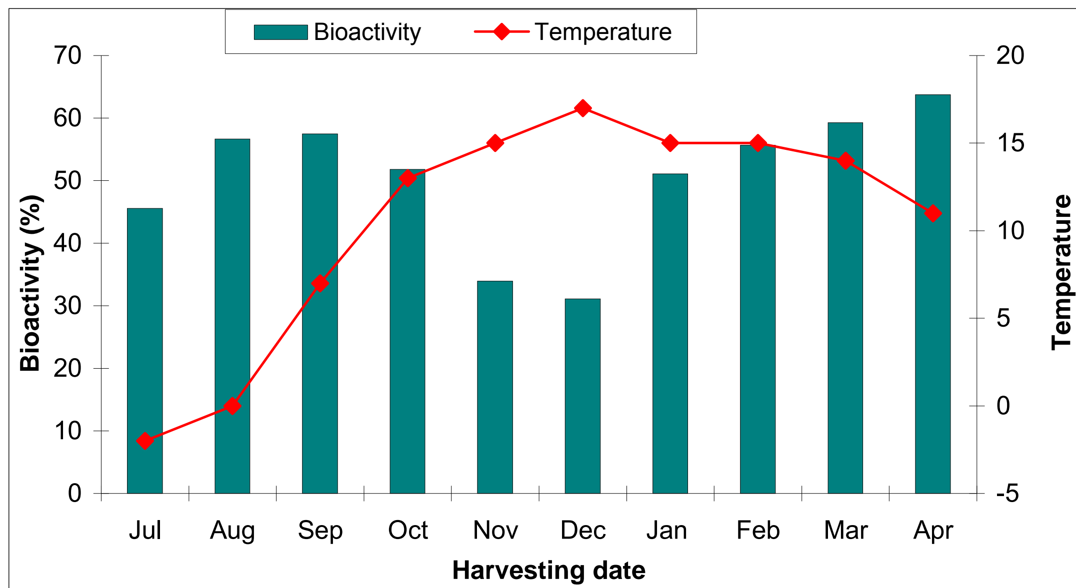


Figure 6.8 Bioactivity and night temperature on *T. violacea* bulb extracts obtained from the autumn planting

Results obtained from the spring planting season indicated that better bioactivity was obtained during cooler months (June, July, August). Results from both plantings (Figures 6.8 and 6.9) indicated that during warmer months bioactivity was generally low but increased as night temperatures started to decrease (Tables 6.1 and 6.2). The highest percentage bioactivity in the spring planting season was obtained in the August 2007 sampling date (Figure 6.9). Plant restores energy during winter in preparing to grow during suitable climatic conditions.

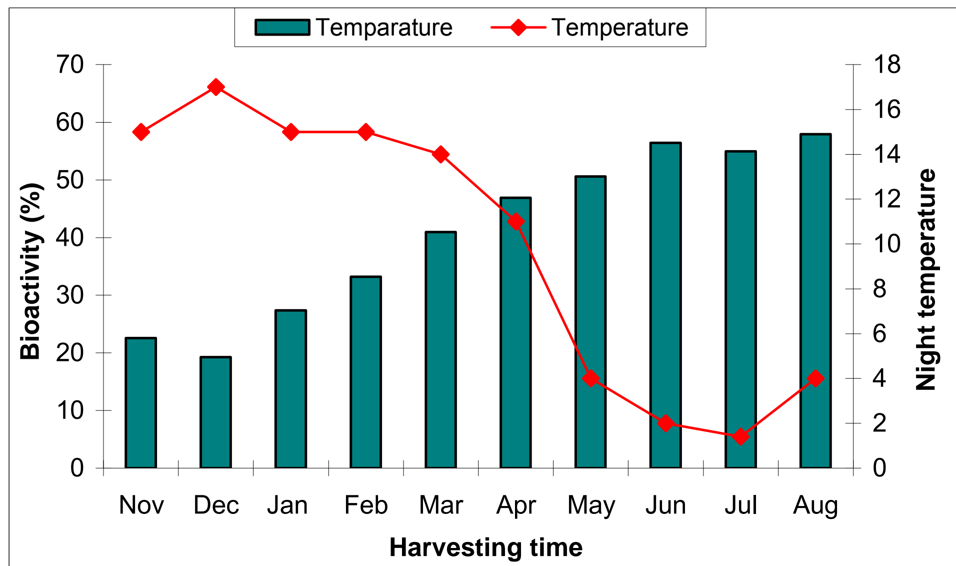


Figure 6.9 Bioactivity and night temperature on *T. violacea* bulb extracts obtained from the winter planting

## 6.5 CONCLUSIONS

Findings of this study showed that autumn planting season adversely affected plant growth, yield and bioactivity of *T. violacea* plants. *T. violacea* plants are sensitive to a very low temperature of below 0°C. *T. violacea* is recommended to be planted during late winter months since plant establishment is good during the subsequent spring season. Bioactivity was also found to be better during winter when the minimum temperatures are low. During summer months, *T. violacea* plants produced a low bioactivity percentage due to the fact that plants were actively flowering. Therefore, late winter planting is recommended for *T. violacea* in order to obtain higher yield and better bioactivity.

## 6.6 SUMMARY

*T. violacea* bulbs were planted in autumn (April 2006) and in winter (August 2006) at the Experimental Farm of the Agricultural Research Council at Roodeplaat. The two experiments were identical in terms of experimental layout and treatments applied. Plants were harvested every month from the fourth

month after planting for a total of ten months. Growth analysis and screening of bioactivity were conducted at each monthly sampling. The total yield was also computed at the end of the growing season when all plants were harvested from the plots.

Establishing *T. violacea* plants during autumn lead plants to grow slowly though-out the cold winter months. Plants that were planted in winter established very fast during spring and summer months. Comparing yields obtained from two planting seasons indicated that the total yields that were harvested from winter planting were higher than the one of autumn planting season. Higher bioactivity was also obtained from the winter planting season during cooler months when compared with autumn planting season. *Tulbaghia violacea* plant is, therefore, recommended for winter planting in order to obtain higher yield and better bioactivity for use as a medicinal plant.

# CHAPTER 7

# INHIBITORY EFFECTS OF WILD GARLIC (*TULBAGHIA VIOLACEA* HARV.) AND EGYPTIAN WHITE GARLIC (*ALLIUM SATIVUM* L.) PLANT EXTRACTS ON THE GROWTH OF *SCLEROTIUM ROLFSII* AND *ALTERNARIA SOLANI*

## 7.1 INTRODUCTION

Potato (*Solanum tuberosum* L.) and tomato (*Lycopersicon esculentum* Mill.) are the most important vegetable crops grown in South Africa. These crops are grown almost throughout the year in fields and in hydroponics systems. Yield loss of both potato and tomato is associated with fungal infections. Diseases mainly affect the aerial part of tomato and potato plants as well as the stem, fruit and roots (Floolad & Ntahimpera, 2000; Batista, Lima, Haddad, Maffia & Mizubuti, 2006). Fungal diseases cause vascular wilt by infecting plants through the leaves by growing internally through the cortex to the branches and stems.

*Alternaria solani* and *Sclerotium rolfsii* are controlled mainly by large amounts of chemicals to protect and prevent crop losses. Fungicides are sprayed every 7-10 days or twice a week, regardless of weather conditions (Batista *et al.*, 2006). The applied chemicals are dangerous to the environment, grazing animals and human health (Nteso & Pretorious, 2006).

It is important to introduce a new management strategy to lower the amount of fungicides applied to the crops by means of plant extracts. The adoption of integrated disease management can work to minimize the use of chemicals. The aim of study was to determine the minimum inhibitory concentration of *A. sativum* and *T. violacea* bulb extracts against *A. solani* and *S. rolfsii* plant pathogens.

## 7.2 MATERIALS AND METHODS

The study was conducted at the Experimental Farm of the Agricultural Research Council - Vegetable and Ornamental Plant Institute (ARC-VOPI) at Roodeplaat, Pretoria. Plant materials that were collected from three experiments (discussed in Chapters 2, 4, and 6) were screened to determine the minimum inhibitory concentration of both *A. sativum* and *T. violacea* plants.

*A. sativum* plants that were harvested at 175 days after planting (DAP) and were treated with ammonium sulphate at 100 $kg{\cdot}ha^{-1}$ were used to determine the minimum inhibitory concentration (MIC). *T. violacea* plants that were treated with 100 $kg{\cdot}ha^{-1}$ ammonium sulphate and harvested at the end of the growing period were used to determine the minimum inhibitory concentration. Plant materials were dried in an air-forced oven at 40 ºC for two weeks and thereafter milled.

Plant extracts were prepared from bulbs according to the method described by Rios *et al.* (1988). Thirty grams of powdered plant material were extracted with methanol. Bottles were placed on a shaking machine for 24 hours, after which they were filtered under vacuum through Whatman filter paper (No.1). The filtrates were poured into 90 mm petri dishes which were left open to allow the methanol to evaporate over night. After drying, the plant extracts were stored at -20 ºC.

Antifungal activity was qualitatively evaluated by means of the agar plate diffusion assay technique (Rios *et al*., 1988). Malt agar was used to prepare mother cultures of two plant pathogens (*S. rolfsii* and *A. solani*) obtained from the Plant Protection Research Institute of the Agricultural Research Council (ARC) in Pretoria, South Africa. Two mother cultures were prepared for each fungus, with one being a working culture and the other a back-up culture stored in a refrigerator at 4-10 ºC.

Two plant pathogenic fungi were cultured on 0.05% malt agar that was prepared according to the instructions from Merck (Nteso & Pretorius, 2006). The malt agar solution was autoclaved for 20 minutes at 125 ºC and then cooled to 45 ºC in a water bath. An amount of 33% (m/v) streptomycin solution was added to the basal medium to control bacterial growth. A methanol extract was dissolved in 0.3 mL sterile distilled water. Bulb crude extracts were tested in a concentration range (0.006, 0.01, 0.03, 0.05, 0.1, 0.2, 0.4, 0.6, 0.8, 1, 1.5 and 2 mg·mL$^{-1}$) to determine the MIC. The medium was poured into 65 mm petri dishes and allowed it to set under sterile conditions. The centre of each petri dish was inoculated with a 6 mm diameter plug obtained from the mother culture. A petri dish containing only the basal medium served as a control. Petri dishes containing tebuconazole/triazole (folicur, 250g/LEW) were used as standard fungicides against each test in order to determine the effectiveness of the plant extracts. Each treatment was replicated four times. The plates were incubated for 2 days at 25 ºC in a growth cabinet.

Results were statistically analyzed using GenStat, 2005. Differences between treatments were tested by means of analysis of variance (ANOVA).

## 7.3 RESULTS AND DISCUSSION

### 7.3.1 Effect of *A. sativum* against *S. rolfsii* and *A. solani* pathogens

*A. sativum* and *T. violacea* are known to have antibacterial, antiviral and antifungal activities (Bakri & Douglas, 2005), but in this study they were assessed for their activity against two plant pathogenic fungi (*A. solani* and *S. rolfsii*). The results indicated that the MIC effects of *A. sativum* against *S. rolfsii* and *A. solani* were at 0.01 mg·mL$^{-1}$ and the maximum of *S. rolfsii* was at 0.8 mg·mL$^{-1}$ while the maximum for *A. solani* was at 1 mg·mL$^{-1}$ (Figures 7.1). There were no inhibitions of *S. rolfsii* and *A. solani* at 0.006 mg·mL$^{-1}$ concentrations. *S. rolfsii* was more sensitive to plant extracts than *A. solani.*

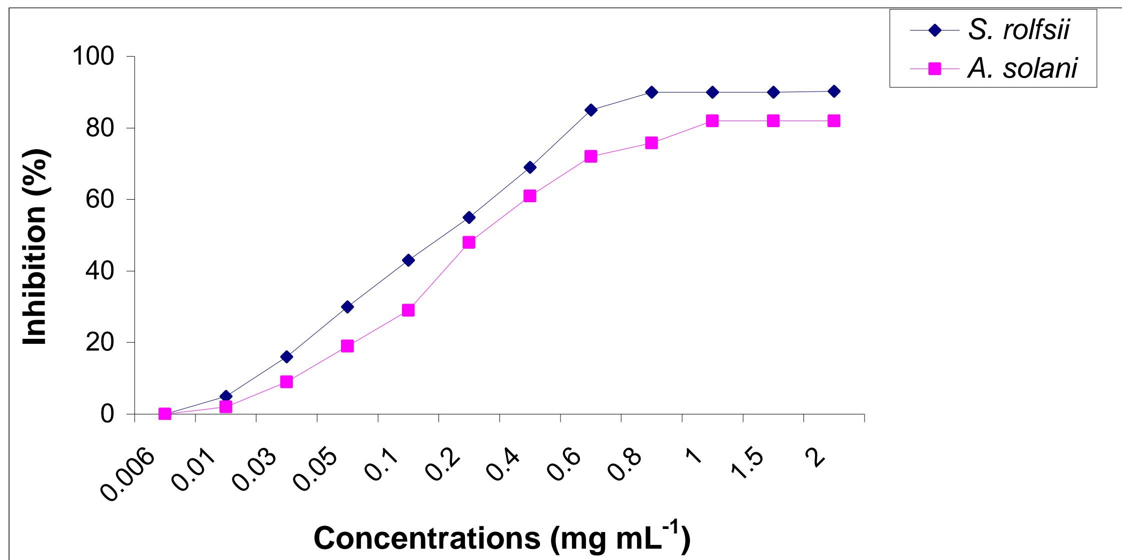


Figure 7.1 Inhibitory effects of *A. sativum* plant extracts against *A. solani* and *S. rolfsii*

Figures 7.2 A and B indicate the inhibition effects of *A. sativum* against *A. solani* plant pathogen. In Figure 7.2 B, there were no differences between plant extracts and standard fungicides, when compared to the control. Both fungicides and plant extracts inhibited the growth of *A. solani* when applied at the same rate.

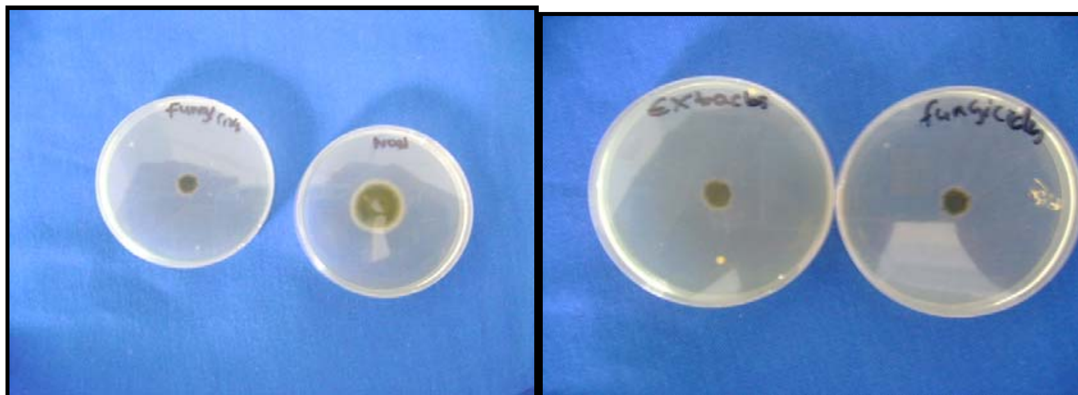

Figure 7.2 Inhibitory effects of *A. sativum* extracts and fungicides against *A. solani*. A1: *A. solani* that was treated by a standard fungicide. A2: *A. solani* that was not treated (control). B1: *A. solani* treated with *A. sativum* plant extract. B2: *A. solani* that was treated by a standard fungicide

### 7.3.2 Effect of *T. violacea* against *S. rolfsii* and *A. solani* pathogens

Results obtained from this study indicated that the MIC of *T. violacea* extracts against *A. solani* were at 0.006 mg·mL$^{-1}$ and the maximum at 1 mg·mL$^{-1}$. *S. rolfsii* was more sensitive to *T. violacea* extracts and its MIC was found at 0.006 mg·mL$^{-1}$ but with a very low percentage of inhibition, while the maximum inhibitory concentrations was 0.6 mg·mL$^{-1}$ (Figures 7.3).

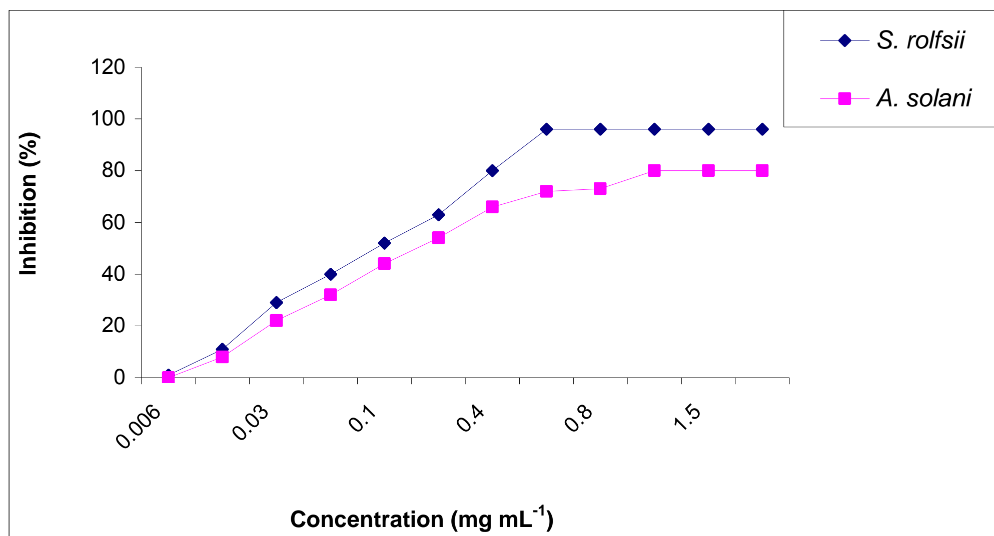


Figure 7.3 Inhibitory effects of *T. violacea* plant extracts against *A. solani* and *S. rolfsii*

Figures 7.4 A and B indicate the inhibition effects of *T. violacea* against *S. rolfsii* plant pathogen. In Figure 7.4 B there were no differences between plant extracts and standard fungicides, when compared to the control. Both fungicides and plant extracts inhibited the growth of *S. rolfsii* when applied at the same rate.

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Figure 7.4 Inhibitory effects of *T. violacea* extracts and fungicides against *S. rolfsii*. A1: *S. rolfsii* that was treated by a standard fungicide. A2: *S. rolfsii* that was not treated (control). B1: *S. rolfsii* treated with *T. violacea* plant extract. B2: *S. rolfsii* that was treated by a standard fungicide

The MIC of the results obtained from autumn and winter planting seasons were similar. The results for *S. rolfsii* were better when compared with the findings of Nteso & Pretorious (2006). Nteso & Pretorious (2006) found that at a concentration of 1 $mg \cdot mL^{-1}$, *T. violacea* plant extracts were significantly higher than the synthetic fungicides against *S. rolfsii*.

The findings of this study are supported by the results obtained by Lee, Chang, Su, Huang & Jang (2007) who stated that the non-treated fungi were abundant and having a clear appearance, while the growth of plant pathogenic fungi treated with plant extracts were remarkably decreased (Figure 7.2 & 7.4). The findings from this study confirm that *A. sativum* and *T. violacea* can be used as fungicides against *S. rolfsii* and *A. solani* pathogens. *A. sativum* and *T. violacea* plants can be used to control or prevent the growth of human fungal species and these two plant species can potentially be used as antibiotics to prevent fungal infections in human beings.

## 7.4 CONCLUSIONS

The objective of the study was to determine the minimum inhibitory concentration of *A. sativum* and *T. violacea* bulb extracts against *A. solani* and *S. rolfsii* plant pathogens. *A. sativum* extract failed to inhibit the growth of *A. solani* and *S. rolfsii* at a concentration of 0.006 mg·mL$^{-1}$. The MIC effects of *A. sativum* against *S. rolfsii* and *A. solani* were at 0.01 mg·mL$^{-1}$ and the maximum effects were at 0.8 mg·mL$^{-1}$ concentration, while the maximum effects for *A. solani* were at 1 mg·mL$^{-1}$.

The MIC effects of *T. violacea* extracts against *A. solani* were at 0.006 mg·mL$^{-1}$ and the maximum effects were at 1 mg·mL$^{-1}$. The MIC of *S. rolfsii* were found at 0.006 mg·mL$^{-1}$ and the maximum inhibitory concentrations were at 0.6 mg·mL$^{-1}$. The MIC of *T. violacea* extracts were better when compared with the findings of other scientists. Plants that were treated with ammonium sulphate fertilizer at a level of 100 kg·ha$^{-1}$ were more effective to control the growth of *A. solani* and *S. rolfsii* pathogens.

## 7.5 SUMMARY

The study was conducted to determine the MIC of *A. sativum* and *T. violacea* bulb extracts against *A. solani* and *S. rolfsii* plant pathogen. It was achieved by screening *A. sativum* plants that were harvested at 175 DAP and been treated with ammonium sulphate at 100 kg·ha$^{-1}$. Whereas *T. violacea* plants that was used were treated with 100 kg·ha$^{-1}$ ammonium sulphate and harvested at the end of the growing period. The MIC effects of *A. sativum* against *S. rolfsii* and *A. solani* were at 0.01 mg·mL$^{-1}$ and the maximum were at 0.8 mg·mL$^{-1}$ concentration and the maximum of *A. solani* were at 1 mg·mL$^{-1}$. The MIC of *T. violacea* extracts against *A. solani* was at 0.006 mg·mL$^{-1}$ and the maximum at 1 mg·mL$^{-1}$. These results suggest that *A. sativum* and *T. violacea* extracts that was supplied with ammonium sulphate can be used as a potential alternative to synthetic fungicides to increase the control of fungal pathogens.

## GENERAL DISCUSSION AND CONCLUSIONS

*A. sativum* and *T. violacea* are amongst the most important medicinal plants used by South African traditional healers for the treatment of flu, fever, cold, tuberculosis, cancer of the oesophagus, asthma and many other diseases. Demand for medicinal plants has lead to increasing pressure on wild plant populations. The heavy demand for *T. violacea* could result in local extinction (Netshiluvhi, 1996) if cultivation of the plants is not done. However, growth, yield and quality are constrained by excessive and under fertilization. Investigations by Potgieter (2006) found that excessive application of nitrogen fertilizer lead to poor plant production in terms of both yield and quality.

There is little information on fertilization of *A. sativum* and *T. violacea* to improve plant production. Therefore, the study was carried out to determine:

- The effect of N sources (ammonium sulphate and calcium nitrate) on yield and quality of *Allium sativum* and *T. violacea* plants;
- The best season for harvesting *T. violacea*;
- The antifungal effects of *A. sativum* and *T. violacea* plant extracts against plant pathogens *Altenaria solani* and *Sclerotium rolfsii*.

In general, both nitrogen sources improved fresh leaf and bulb mass and leaf area of *A. sativum* plants. Highest leaf N percentage was obtained with *A. sativum* leaves that were treated with 150 and 200 $kg{\cdot}ha^{-1}$ N as ammonium sulphate fertilizer 140 days after planting (DAP) and with 200 $kg{\cdot}ha^{-1}$ N as ammonium sulphate for bulbs that were harvested 175 DAP. Highest S percentage was obtained at 82 DAP in *A. sativum* leaves that were treated with 200 $kg{\cdot}ha^{-1}$ N from ammonium sulphate. Plants treated with calcium nitrate produced the highest leaf N percentage at 112 DAP and the highest bulb N percentage was found 175 DAP. The application of calcium nitrate resulted in an increase in marketable yields ($t{\cdot}ha^{-1}$) with increased application of N up to 150 $kg{\cdot}ha^{-1}$. This trend changed at 200 $kg{\cdot}ha^{-1}$ where yield decreased. The greatest *A. sativum* bulb mass of 128.8 g per plant was obtained with 150 $kg{\cdot}ha^{-1}$ N as

calcium nitrate. Therefore, it can be concluded that the highest yield was produced from the plants treated with calcium nitrate at 150 kg·ha$^{-1}$ N that gave a yield of 24 t·ha$^{-1}$. Results indicated that *A. sativum* plants treated with ammonium sulphate produced the highest yield of 27 t·ha$^{-1}$ at 200 kg·ha$^{-1}$ N and ammonium sulphate can thus be recommended as a better nitrogen source over calcium nitrate.

A correlation was found from this study between the sampling date and the percentage bioactivity of *A. sativum* leaves and bulb parts. Ammonium sulphate fertilizer improved bioactivity of leaves and the highest bioactivity was produced at 82 and 112 DAP. After 112 DAP leaf bioactivity started to decrease, and by then leaves were growing older and drying off. Ammonium sulphate increased the production of bioactivity compared to plants supplied with calcium nitrate. Bulbs produced very poor bioactivity during the early stages of plant growth. According to the results, the higher the levels of calcium nitrate applied to the plants, the lower was the bioactivity produced. Ammonium sulphate is thus a better source of nitrogen fertilizer. Findings of this study revealed that calcium nitrate failed to improve the medicinal properties of *A. sativum* plant.

Nitrogen source affected plant growth and yield of *T. violacea* plants. Untreated plants struggled to grow due to lack of nitrogen nutrition and resulted in poor plant growth and low yield. An increase in nitrogen nutrition resulted in increases in plant height, leaf area, new tillers, dry leaf and bulb mass, as well as yield. Results of this study indicated that more nitrogen accumulated in the plant leaves than in bulbs and the same applied to sulphate accumulation. There were no interactions between calcium nitrate and ammonium sulphate for yield produced in tons per hectare. Yields that were produced by both fertilizers were not significantly different. Ammonium sulphate fertilizer produced the highest yield of 23.6 t·ha$^{-1}$ while 23.5 t·ha$^{-1}$ was produced from plants supplied with calcium nitrate fertilizer (Table 4.2).

The crude extracts of *T. violacea* bulbs that were treated with ammonium sulphate significantly inhibited the growth of plant pathogenic fungi, *S. rolfsii* and *A. solani* whereas extracts from plants treated with calcium nitrate actually showed a decrease in bioactivity. The results obtained from plants treated with calcium nitrate indicated an improvement in plant yield but with very low bioactivity. Therefore calcium nitrate could be used in the production of *T. violacea* that is planted for horticultural purposes and not for medicinal use.

Autumn and spring planting seasons affected plant growth, yield and bioactivity of *T. violacea* plants. Autumn planting season affected plant development because of low minimum temperatures of about -2 and -3 ºC (Table 6.1) that shocked the plants before proper plant establishment. Spring planting season resulted in higher total yield due to a higher number of new tillers that were produced throughout the growing season. Better bioactivity was also obtained during winter when the minimum temperatures were low. In December month, minimum temperatures increased and *T. violacea* plants failed to produce good bioactivity. *T. violacea* is recommended to be grown during late winter months so that it can establish well during spring season.

Plants that were treated with ammonium sulphate fertilizer at 100 $kg \cdot ha^{-1}$ N were more effective in controlling the growth of *A. solani* and *S. rolfsii* pathogens. The minimum inhibitory concentration (MIC) effects of *A. sativum* against *S. rolfsii* and *A. solani* were at 0.01 $mg \cdot mL^{-1}$ and the maximum effects against *S. rolfsii* were at 0.8 $mg \cdot mL^{-1}$, while the maximum effects against *A. solani* were at 1 $mg \cdot mL^{-1}$. The MIC effects of *T. violacea* extracts against *A. solani* were at 0.006 $mg \cdot mL^{-1}$ and the maximum effects were at 1 $mg \cdot mL^{-1}$. The MIC effects against *S. rolfsii* were found at 0.006 $mg \cdot mL^{-1}$ and the maximum inhibitory concentrations at 0.6 $mg \cdot mL^{-1}$. The MIC of *T. violacea* extracts were higher when compared with findings of other scientists which was 1 $mg \cdot mL^{-1}$.

Sufficient data is provided in this study to suggest that further testing of human pathogenic fungi can be used. The use of *A. sativum* and *T. violacea* plant

extracts as fungicide against *S. rolfsii* and *A. solani* is recommended as treatments on tomato and potato. With more research, no doubt *A. sativum* and *T. violacea* plant extracts will ultimately be allowed to be registered as fungicide treatments for tomato and potato production in South Africa.

## GENERAL SUMMARY

A study was conducted to determine the effect of N sources (ammonium sulphate and calcium nitrate) on yield and quality of *Allium sativum* and *Tulbaghia violacea*. Furthermore, to determine the best season for harvesting *T. violacea* and to determine the antifungal effects of *A. sativum* and *T. violacea* plant extract against plant pathogens *Altenaria solani* and *Sclerotium rolfsii*. The study was conducted at the Experimental Farm of the Agricultural Research Council - Vegetable and Ornamental Plant Institute (ARC - VOPI) at Roodeplaat, Pretoria. Three experiments were initiated during the period April 2006 until August 2007.

*T. violacea* plants were obtained from Lifestyle Seed Company and *A. sativum* bulbs were obtained from Agribez Company. *A. sativum* and *T. violacea* plants were treated with ammonium sulphate (21% N) and calcium nitrate (16% N) fertilizer applied as topdressing treatments at a total of 0, 50, 100, 150 and 200 $kg{\cdot}ha^{-1}$, divided into three applications at three week (*A. sativum*) and three month (*T. violacea*) intervals.

*A. sativum* plants were sampled at 54, 82, 112, 140 and 175 days after planting (DAP), while *T. violacea* plants were sampled monthly for ten months. The parameters recorded were number of leaves, plant height (cm), fresh and dry leaf mass (g), fresh and dry bulb mass (g), neck and bulb circumference (mm) and leaf area ($cm^2$). Chemical analysis, yield and bioactivity determinations were done on both plant species.

Both nitrogen sources improved fresh leaf and bulb mass, leaf area, of *A. sativum* plants. Most tissue N and S were found in plant leaves than those in bulbs during the early stages of *A. sativum* growth. Thereafter, higher N and S were obtained in bulbs than in leaves of *A. sativum* at the end of the growing season. Yield obtained from plants treated with calcium nitrate peaked at 24 $t{\cdot}ha^{-1}$, while for plants treated with ammonium sulphate, yield peaked at 27 $t{\cdot}ha^{-1}$. *A. sativum* plants that were supplied with ammonium sulphate fertilizer improved

leaf bioactivity for sampling done at 82 to 112 DAP, while bulbs produced higher percentage bioactivity at 175 DAP (maturity). Ammonium sulphate increased the production of bioactivity in plants compared to calcium nitrate. *A. sativum* bulbs produced very poor bioactivity during the early stages of plant growth. Ammonium sulphate was a better source of nitrogen since calcium nitrate failed to improve the medicinal properties of *A. sativum*.

Nitrogen source affected plant growth and yield of *T. violacea* plants. An increase in nitrogen nutrition resulted in an increase in plant height, leaf area, new tillers, dry leaf and bulb mass, as well as yield. More nitrogen accumulated in plant leaves than in bulbs and similarly for sulphate accumulation. Ammonium sulphate fertilizer produced the highest yield of 23.6 $t{\cdot}ha^{-1}$ which was similar to 23.5 $t{\cdot}ha^{-1}$ from calcium nitrate fertilizer. Bioactivity obtained from *T. violacea* bulbs that were treated with ammonium sulphate was higher than those treated with calcium nitrate. Calcium nitrate could, however, be used in the production of *T. violacea* that is planted for horticultural purposes and not for medicinal use.

The effects of winter or autumn planting season were evident for plant growth parameters, yield and bioactivity of *T. violacea* plants. Autumn planting season negatively affected plant development because of low minimum temperatures of about -3 and -2ºC that shocked the plants before proper plant establishment. Spring to spring planting season resulted in higher total yield due to a higher number of new tillers that were produced throughout the growing season. Higher bioactivity percentage was also obtained during winter when the minimum temperatures were low.

Both *A. sativum* and *T. violacea* plants that were treated with ammonium sulphate fertilizer at 100 $kg{\cdot}ha^{-1}$ N were more effective in controlling the growth of *A. solani* and *S. rolfsii* pathogens. The MIC effects of *A. sativum* against *S. rolfsii* and *A. solani* were at 0.01 $mg{\cdot}mL^{-1}$ and the maximum concentration effects against *S. rolfsii* were at 0.8 $mg{\cdot}mL^{-1}$ while the maximum effects against *A. solani* were at 1 $mg{\cdot}mL^{-1}$. The MIC effects of *T. violacea* extracts against *A. solani* were

at 0.006 $mg{\cdot}mL^{-1}$ and the maximum effects were at 1 $mg{\cdot}mL^{-1}$. The MIC effects against *S. rolfsii* were found at 0.006 $mg{\cdot}mL^{-1}$ and the maximum inhibitory concentrations were at 0.6 $mg{\cdot}mL^{-1}$. This study revealed that *A. sativum* and *T. violacea* plants contain the bioactivity that inhibits the growth of plant pathogenic fungi *S. rolfsii* and *A. solani*. Both plants contain one of these natural compounds that has been identified as allicin, an extracts that accumulated in, due to their high organo-sulphur compounds content. That is a sulphur-containing constituents in *Allium* crops are the *S*-alk(en)yl-L-cysteine suphoxides. Therefore, these plant extracts (allicin) can be used to inhibit human pathogenic fungi and those two plant species can potentially be used as an antibiotic to prevent fungal infections in human beings.

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# APPENDICES

## APPENDIX SOIL STATUS BEFORE PLANTING

Table A 1 Chemical analysis of soil at ARC-VOPI Experimental Farm before planting, April/August 2006/7

| Depth (cm) | Phosphorus ($mg \cdot kg^{-1}$) | Potassium ($mg \cdot kg^{-1}$) | Calcium ($mg \cdot kg^{-1}$) | Magnesium ($mg \cdot kg^{-1}$) | Magnesium ($mg \cdot kg^{-1}$) | Sodium ($mg \cdot kg^{-1}$) | Nitrogen (%) | Sulphur ($mg \cdot kg^{-1}$) | Soil pH |
|---|---|---|---|---|---|---|---|---|---|
| 0-30 | 78.90 | 178 | 1330 | 456 | 61 | 0.05 | 117.74 | 117.7 | 7.49 |
| 30-60 | 55.10 | 127 | 1486 | 502 | 66 | 0.03 | 110.46 | 110.5 | 7.60 |

## APPENDIX A: FIELD EXPERIMENTS

Table A 2 Analysis of variance for leaf and bulb characteristics of *A. sativum* plants as affected by nitrogen application

| Sources of variation | Df | Mean Squares[z] | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | Plant height (cm) | Leaf area (cm) | Neck circumferences (mm) | Leaf tissue nitrogen (%) | Bulb tissue nitrogen (%) | Leaf tissue sulphur (%) | Bulb tissue sulphur (%) |
| | | | | | *54 Days After Planting* | | | |
| Treatment | 8 | 14.83** | 478.30** | 31.80* | 0.02** | 0.02** | 0.01** | 0.00** |
| Replication | 3 | 1.36 ns | 11.01 ns | 1.80 ns | 0.01 ns | 0.05 ns | 0.02 ns | 0.01 ns |
| Error | 35 | 3.63 | 16.23 | 5.54 | 0.06 | 0.11 | 0.08 | 0.05 |
| | | | | | *82 Days After Planting* | | | |
| Treatment | 8 | 35.83** | 3301.00** | 58.30* | 0.04** | 0.02** | 0.01** | 0.00** |
| Replication | 3 | 2.46 ns | 17.56 ns | 1.57 ns | 0.02 ns | 0.06 ns | 0.01 ns | 0.00 ns |
| Error | 35 | 5.26 | 37.68 | 4.71 | 0.11 | 0.03 | 0.04 | 0.06 |
| | | | | | *112 Days After Planting* | | | |
| Treatment | 8 | 64.39** | 8472.00** | 35.60* | 0.64** | 0.04** | 0.05** | 0.01** |
| Replication | 3 | 3.88 ns | 54.98 ns | 2.37 ns | 0.29 ns | 0.07 ns | 0.03 ns | 0.00 ns |
| Error | 35 | 4.82 | 57.88 | 3.98 | 0.35 | 0.14 | 0.10 | 0.05 |
| | | | | | *140 Days After Planting* | | | |
| Treatment | 8 | 127.27* | 22519.00** | 33.70* | 0.77** | 1.37** | 0.01** | 0.01** |
| Replication | 3 | 2.59 ns | 31.70 ns | 2.21 ns | 0.16 ns | 0.31 ns | 0.01 ns | 0.01 ns |
| Error | 35 | 4.16 | 81.80 | 3.77 | 0.51 | 0.57 | 0.11 | 0.08 |
| | | | | | *175 Days After Planting* | | | |
| Treatment | 8 | 237.93* | 22361.00** | 32.80* | 0.28** | 0.61** | 0.00** | 0.03** |
| Replication | 3 | 1.46 ns | 29.20 ns | 1.96 ns | 0.11 ns | 0.28 ns | 0.01 ns | 0.03 ns |
| Error | 35 | 3.94 | 76.30 | 4.88 | 0.33 | 0.53 | 0.11 | 0.14 |

[Z]F-values significant (*), highly significant (**) or non-significant (NS) at 0.05 level of probability

Table A 3 Analysis of variance for effect of calcium nitrate and ammonium sulphate on *A. sativum* number of cloves, neck and bulb circumference and bulb mass

| Sources of variation | Df | Mean Squares$^{z}$ | | | |
|---|---|---|---|---|---|
| | | Neck circumference (mm) | Bulb circumference (mm) | Clove number (no./bulb) | Bulb mass (g) |
| Calcium nitrate (C) | 4 | 167.57** | 3343.17** | 178.97* | 2001.07** |
| Amm. Sulphate (A) | 4 | 119.08** | 1827.33** | 119.77** | 961.64** |
| C.A | 8 | 114.63** | 1815.53** | 112.00* | 1235.52** |
| Replication | 3 | $1.53^{ns}$ | $2.45^{ns}$ | $0.69^{ns}$ | $1.76^{ns}$ |
| Error | 35 | 3.95 | 6.95 | 1.89 | 6.81 |

$^{Z}$F-values significant (*), highly significant (**) or non-significant (NS) at 0.05 level of probability

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Table A 4 Analysis of variance for effect of ammonium sulphate on *T. violacea* leaves and bulbs sampled from July 2006 to April 2007

| Sources of variation | Df | Mean Squares$^{z}$ | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | July | August | September | October | November | December | January | February | March | April |
| | | *Leaf tissue sulphur (%)* | | | | | | | | | |
| Treatment | 4 | 0.01$^{ns}$ | 0.10$^{ns}$ | 0.12* | 0.02* | 0.01$^{ns}$ | 0.12* | 0.02$^{ns}$ | 0.01$^{ns}$ | 0.06$^{ns}$ | 0.19* |
| Replication | 3 | 0.01$^{ns}$ | 0.01$^{ns}$ | 0.03$^{ns}$ | 0.01$^{ns}$ | 0.00$^{ns}$ | 0.01$^{ns}$ | 0.02$^{ns}$ | 0.01$^{ns}$ | 0.06$^{ns}$ | 0.01$^{ns}$ |
| Error | 12 | 0.16 | 0.08 | 0.16 | 0.06 | 0.06 | 0.04 | 0.14 | 0.13 | 0.21 | 0.13 |
| | | *Bulb tissue sulphur (%)* | | | | | | | | | |
| Treatment | 4 | 0.02$^{ns}$ | 0.19* | 0.23* | 0.02$^{ns}$ | 0.01$^{ns}$ | 0.01* | 0.01$^{ns}$ | 0.01$^{ns}$ | 0.03$^{ns}$ | 0.06$^{ns}$ |
| Replication | 3 | 0.01$^{ns}$ | 0.01$^{ns}$ | 0.04$^{ns}$ | 0.01$^{ns}$ | 0.00$^{ns}$ | 0.01$^{ns}$ | 0.02$^{ns}$ | 0.13$^{ns}$ | 0.04$^{ns}$ | 0.01$^{ns}$ |
| Error | 12 | 0.12 | 0.12 | 0.10 | 0.12 | 0.11 | 0.05 | 0.10 | 0.09 | 0.16 | 0.17 |

$^{z}$F-values significant (*), highly significant (**) or non-significant (NS) at 0.05 level of probability

Table A 5 Analysis of variance for effect of ammonium sulphate on *T. violacea* leaves and bulbs sampled from July 2006 to April 2007

| Sources of variation | Df | Mean Squares[z] | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | July | August | September | October | November | December | January | February | March | April |
| | | | | | | *Leaf tissue nitrogen (%)* | | | | | |
| Treatment | 4 | 0.07* | 1.05* | 3.18** | 0.75* | 0.36** | 0.57 | 0.73* | 2.30** | 2.93* | 2.16** |
| Replication | 3 | 0.04 ns | 0.05 ns | 0.04 ns | 0.31 ns | 0.01 ns | 0.12 ns | 0.13 ns | 0.11 ns | 0.11 ns | 0.05 ns |
| Error | 12 | 0.24 | 0.36 | 0.19 | 0.26 | 0.27 | 0.23 | 0.31 | 0.13 | 0.26 | 0.14 |
| | | | | | | *Bulb tissue nitrogen (%)* | | | | | |
| Treatment | 4 | 0.02 ns | 0.17** | 0.05 ns | 0.07 ns | 0.03 ns | 0.09 ns | 0.06 ns | 1.62 ns | 0.01 ns | 0.53 ns |
| Replication | 3 | 0.04 ns | 0.03 ns | 0.04 ns | 0.12 ns | 0.01 ns | 0.04 ns | 0.03 ns | 0.01 ns | 0.01 ns | 0.01 ns |
| Error | 12 | 0.17 | 0.15 | 0.14 | 0.38 | 0.12 | 0.28 | 0.33 | 0.17 | 0.19 | 0.13 |

[z]F-values significant (*), highly significant (**) or non-significant (NS) at 0.05 level of probability

Table A 6 Analysis of variance for effect of calcium nitrate on *T. violacea* leaves and bulbs sampled from July 2006 to April 2007

| Sources of variation | Df | Mean Squares[z] | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | July | August | September | October | November | December | January | February | March | April |
| | | | | | | *Leaf tissue nitrogen (%)* | | | | | |
| Treatment | 4 | 1.24** | 1.25** | 3.41** | 0.81* | 0.44 | 0.66** | 0.62* | 2.20* | 3.35* | 3.18** |
| Replication | 3 | 0.02 ns | 0.13 ns | 0.13 ns | 0.09 ns | 0.03 ns | 0.06 ns | 0.37 ns | 0.04 ns | 0.05 ns | 0.01 ns |
| Error | 12 | 0.18 | 0.17 | 0.09 | 0.22 | 0.18 | 0.24 | 0.19 | 0.24 | 0.29 | 0.19 |
| | | | | | | *Bulb tissue nitrogen (%)* | | | | | |
| Treatment | 4 | 0.46* | 0.92** | 1.74* | 0.47 ns | 0.07* | 0.00 ns | 0.27 ns | 0.01 ns | 1.19 ns | 0.64** |
| Replication | 3 | 0.01 ns | 0.05 ns | 0.04 ns | 0.03 ns | 0.03 ns | 0.08 ns | 0.15 ns | 0.01 ns | 0.11 ns | 0.01 ns |
| Error | 12 | 0.15 | 0.23 | 0.15 | 0.26 | 0.14 | 0.30 | 0.30 | 0.14 | 0.30 | 0.14 |

[z]F-values significant (*), highly significant (**) or non-significant (NS) at 0.05 level of probability

Table A 7 Analysis of variance for leaf, bulb and yield of *T. violacea* plants as affected by autumn planting season, April 2006 to April 2007

| Sources of variation | Df | Mean Squares[z] | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Plant height (cm) | Leaf number | Leaf area ($cm^2$) | Leaf fresh mass (g) | Dry leaf mass (g) | Number of new tillers | Fresh bulb mass (g) | Dry bulb mass (g) | Bulb circumfe rences (mm) | Number of flowers | Yield ($t \cdot ha^{-1}$) |
| Har (H) | 9 | 170.57** | 7304.80** | 5087474.0* | 78498.80* | 981.00** | 2911.00* | 84939.60** | 1691.28* | 4985.91 ns | 9.04* | 217.42* |
| Fer (F) | 9 | 250.46** | 3537.35** | 330285.00* | 6225.00* | 218.00** | 363.00* | 11929.10** | 1042.79* | 944.19 ns | 0.38** | 102.05* |
| Fer.Har (FxH) | 81 | 4.31** | 92.90** | 24720.00* | 324.00* | 7.82** | 27.91* | 349.60* | 159.65* | 27.79 ns | 0.05** | 29.76* |
| Replication | 3 | 3.03 ns | 57.30 ns | 8682 ns | 219.20 ns | 4.55 ns | 3.57 ns | 253.70 ns | 43.18 ns | 9.96 ns | 0.24 ns | 12 ns |
| Error | 269 | 1.74 | 7.57 | 93.18 | 14.8 | 2.13 | 1.89 | 15.92 | 6.57 | 3.15 | 0.49 | 3.55 |

[Z]F-values significant (*), highly significant (**) or non-significant (NS) at 0.05 level of probability

Table A 8 Analysis of variance for leaf, bulb and yield of *T. violacea* plants as affected by spring planting season, August 2006 to August 2007

| Sources of variation | Df | Mean Squares[z] | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Plant height (cm) | Leaf Number | Leaf area ($cm^2$) | Fresh leaf mass (g) | Dry leaf mass (g) | Number Of new tillers | Fresh bulb mass (g) | Dry bulb mass (g) | Bulb circumfe rence (mm) | Number of flowers | Yield ($t{\cdot}ha^{-1}$) |
| Har (H) | 9 | 508.66* | 14434.80** | 5690710.00** | 97126.12** | 847.00** | 4542.00** | 105518.01** | 4565.87** | 3291.96* | 38.29* | 338.12** |
| Fer (F) | 9 | 11.87* | 684** | 58939.00** | 145.20** | 7.04** | 52.41** | 452.50** | 92.01** | 35.15 ns | 48.14* | 29.64* |
| Fer.Hav (FxH) | 81 | 1.89* | 80.5** | 16642.00** | 29.61** | 2.42** | 32.42** | 35.30** | 24.09** | 8.97 ns | 3.97 ns | 21.81* |
| Replication | 3 | 4.6 ns | 164.1 ns | 26738.00 ns | 312.01 ns | 6.1 ns | 59.89 ns | 350.6 ns | 30.05 ns | 83.14 ns | 5.28 ns | 30.32 ns |
| Error | 269 | 2.14 | 12.8 | 163.52 | 17.66 | 2.47 | 7.73 | 18.72 | 5.48 | 9.11 | 2.29 | 5.5 |

[Z]F-values significant (*), highly significant (**) or non-significant (NS) at 0.05 level of probability

## APPENDIX B: LABORATORY EXPERIMENTS

Table B 1 Analysis of variance for the inhibitory effects of *A. sativum* leaf and bulb extracts on growth of plant pathogenic fungi

| Sources of variation | Df | Mean Squares$^{z}$ | |
|---|---|---|---|
| | | Leaf inhibition (%) | Bulb inhibition (%) |
| Pat | 1 | 232.94 $^{ns}$ | 1998.09 $^{ns}$ |
| Fer | 1 | 130438.35* | 44732.25* |
| Fer lev | 4 | 376.45* | 1569.98* |
| Pat.Fer | 1 | 1045.71* | 1823.29* |
| Pat.Ferlev | 4 | 36.64* | 190.57* |
| Fer.Ferlev | 4 | 16095.86** | 4847.68** |
| Pat.Har | 4 | 41.32* | 152.18* |
| Fert.Har | 4 | 2754.57* | 2362.71* |
| Ferlev.Har | 16 | 81.89* | 237.25* |
| Pat.Fer.Ferlev | 4 | 78.91** | 119.43** |
| Pat.Fer.Har | 4 | 30.12** | 196.84** |
| Pat.Ferlev.Har | 16 | 28.31** | 40.26** |
| Fer.Ferlev.Har | 16 | 425.07** | 244.97** |
| Pat.Fer.Ferlev.Har | 16 | 20.22** | 19.54** |
| Error | 285 | 2.43 | 2.71 |

$^{Z}$F-values significant (*), highly significant (**) or non-significant (NS) at 0.05 level of probability

Table B 2 Analysis of variance for the inhibitory effects of ammonium sulphate and calcium nitrate on growth of plant pathogenic fungi

| Sources of variation | Df | Mean Squares[z] | |
|---|---|---|---|
| | | Ammonium sulphate | Calcium nitrate |
| Pat | 1 | 41.67** | 128.58** |
| Ferlev | 4 | 5603.41** | 11362.49** |
| Pat.Ferlev | 4 | 18.05** | 10.37** |
| Pat.Har | 9 | 11.42* | 107.37* |
| Ferlev.Har | 36 | 45.26** | 161.09** |
| Pat.Ferlev. Har | 36 | 6.58** | 22.97** |
| Error | 269 | 2.23 | 2.51 |

[z]F-values significant (*), highly significant (**) or non-significant (NS) at 0.05 level of probability

Table B 3 Analysis of variance for the inhibitory effect of nitrogen source on growth of plant pathogenic fungi

| Sources of variation | Df | Mean Squares[z] |
|---|---|---|
| | | N-Sources |
| Pat | 1 | 158.32 [ns] |
| Ferlev | 9 | 21715.49* |
| Pat.Fer | 9 | 13.95* |
| Pat.Har | 9 | 77.84* |
| Fer.Har | 81 | 142.7** |
| Pat.Fer.Har | 81 | 17.68** |
| Error | 570 | 2.39 |

[z]F-values significant (*), highly significant (**) or non-significant (NS) at 0.05 level of probability