THE ALLELOPATHIC POTENTIAL OF *ARCTOTIS ARCTOTOIDES* (L.f.) O. Hoffm ON SOME VEGETABLES

ABIMBOLA ADESILE BADMUS

Submitted in fulfillment of the requirements for the degree:

DOCTOR OF PHILOSOPHY: BOTANY

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SUPERVISOR: PROF A J AFOLAYAN

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DECLARATION

I, **Badmus AA** declare that the thesis hereby submitted for the degree of Doctor of Philosophy in Botany at the University of Fort Hare, Alice**,** is my own independent work except where due references have been acknowledged. This work has neither been submitted previously nor will be submitted for an award at another University or Institution of Higher Education.

Signature……

Date…….

ABSTRACT

Laboratory and greenhouse experiments were conducted to evaluate the allelopathic effects of the extracts and residue *Arctotis arctotoides* (L.f.) O. Hoffm on selected vegetable crops. The study aimed to address the following specific objectives to (i) examine the ultra structures of the leaf of *A. arctotoides* using the Scanning Electron Microscope (SEM), (ii) carry out comprehensive qualitative and quantitative phytochemical analysis of the root and shoot materials of the plant, (iii) investigate the allelopathic activities of the root and shoot aqueous extracts of *A. arctotoides* at concentrations of 10, 8, 6, 4 and 2 mg/ml on germination, radicle and plumule growth of cabbage, carrot, tomato and spinach, (iv) evaluate the inhibitory effects of the dried shoot residue of the plant at 10, 20 and 40 g kg^{-3} of soil (treatments B, C and D) and the control (treatment A) on the morphology, growth and chlorophyll pigment content of tomato and cabbage transplants at 1, 2, 3 and 4 weeks after transplanting and (v) assess the effects of the dried shoot residue of *A. arctotoides* on the yield, nutrient uptake by the leaves of tomato and cabbage at 4 and 12 weeks after transplanting. Finally, to analyze the residual mineral content of the soils with tomato and cabbage transplants at 12 weeks after transplanting. The the SEM revealed that anisocytic stomata and glandular trichomes (GTs) were numerous on the abaxial than the adaxial surfaces of *A. arctotoides*. The non glandular trichomes (NGTs) were also present on both surfaces but lesser on the abaxial. Morphologically, the GTs were peltate, uniseriate and globular head while the NGTs were cylindrical and filamentous with variable number of cells at the basal portion.

The energy dispersive X-ray spectroscopy of some crystals showed that Na^{+} Mg^{2+} and Ca^{2+} were the major constituents of the crystal deposit found around the GTs and stomata. The results of the phytochemical composition of the root and shoot extracts of *A. arctotoides* confirmed the occurrence of alkaloids, flavonoids, phenolics, saponnins, tannins and triterpenes as the common constituents. In addition, cardiac glycosides and steroids were also detected in the shoot of the *A. arctotoides.* Quantitative estimation of the chemical constituents of the crude extracts further revealed that the alkaloid content in the root higher (0.97%) than the shoot (0.64%). The quantity of flavonoids detected in the shoot (1.02%) was more than that observed in the root (0.35%). Others (phenolics and tannins) were marginal in the two plant parts. The results of the inhibitory effects of the root and shoot aqueous extract at the varying concentrations showed that root extract at 10 mg/ml considerably reduced the germination of cabbage, carrot, tomato and spinach seeds by 84.0, 83.2, 72.8 and 37.4% respectively. Incubation of the shoot extract at the same concentration resulted in 100% inhibition of cabbage and carrot seed germination whereas those of tomato and spinach were suppressed by 91.5 and 61.2% respectively. The two extracts at the varying concentrations also had a significant reduction on the radicle and plumule growth of the four vegetables. Addition of the shoot residue to the soil showed massive chlorosis, necrotic lesions and wilting of tomato and cabbage leaves under treatments C and D at 2 weeks after transplanting. The number of leaves, leaf area, dry shoot and root weight of the two vegetables grown in the amended soils were also drastically reduced. The inhibition percentages due to the addition of the three concentrations of *A. arctotoides* dried shoot residue on the dry shoot weight at 4 weeks after transplanting were 38.6, 45.5 and 70.3. for tomato and 57.5, 73.3 and 87.5% for cabbage.

Similarly, the declines in the dry root weight of 61.3, 82.9.4 and 83.4% for tomato as well as 53.1, 54.7 and 67.2% for cabbages were recorded for the two vegetables under treatment B, C and D during the period. The results further showed that the dry fruit yield and shoot weight of tomato under the treatments B, C and D decreased with increase in shoot residue concentrations of *A. arctotoides.* Relative to treatment A, no significant differences were recorded in the dry head weight of cabbage under the residue treated groups. The reductions in the fruit yield and fresh head weight caused by treatments C and D were 37.2 and 84.8% for tomato and 30.9 and 72.4% for cabbage. The findings on the mineral contents in the leaves of the two vegetables revealed significant differences in the uptake of N, Mg, Na, Cu and Fe by tomato leaves. The concentrations of N, K, Na and Zn in cabbage leaves also differed. However, the P content was relatively constant in the leaves of the two vegetables at 4 and 12 weeks after transplanting. At 12 weeks after transplanting, the Fe content in soils with tomato and cabbage treatments C and D was greatly enhanced in comparison with the other nutrients. The residual N, P and Zn detected in soils planted to cabbage were similarly equal among all the groups including the control. Thus, under the greenhouse experiment, *Arctotis arctotoides* (L.f) O. Hoffm has been shown to contain some phytotoxic chemical compounds in its root and shoot materials. The compounds either singly or collectively have demonstrated some inhibitory potentials on the germination, growth and yields of cabbage, carrot, tomato and spinach evaluated in this study.

PREFACE

This dissertation is composed of seven chapters. Chapter 1 is the introduction comprising background information, rationale for the study and the objectives to be achieved. Chapter 2 is a general literature review which establishes the context of allelopathic studies. The review focuses on the complementarities between the inhibitory and stimulatory influences of allelopathy in relation to the phytotoxic activities of the various allelochemicals in the different plant parts. The assessment further considered dose response and sensitivity of the different organisms in addition to various bioassay techniques including laboratory, greenhouse and field experiments. The next four chapters constitute the body of the thesis. Chapter 3 briefly discusses the medicinal herb (*A. arctotoides*) and the four vegetables namely cabbage, carrot, spinach and tomato used in this study. Chapter 4 gives comprehensive details of materials and methods of experiments 1 to 6 conducted during the study while chapter 7 gives summary and conclusion of the findings.

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DEDICATION

This work is dedicated to the Almighty Allah, the most Beneficent, and most Merciful.

AL-HAYUL-QUAYUM, AR-RAHMAN AND AS-SAMAD.

and

My late father, ***Alhaji Okelade Adeniyi Lawal***

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1.0 INTRODUCTION

1.1 Background

Vegetation naturally consists of trees, vines, shrubs and ornamental plants. While many of these plant species provide soil surface cover, prevent erosion, increase soil fertility and soil organic matter, a large number of these species do release several classes of phytotoxic compounds into the rhizosphere. The phytotoxic substances, according to Abreu and Mazzafera (2005) are produced by plants as defensive mechanisms against biotic and abiotic stresses. The occurrence of similar natural products in aquatic lives and microorganisms has also been well documented (Afolayan, 2003, Afolayan et al., 2007; Odeyemi et al., 2008). These organic substances are economically important because of their antimicrobial properties and effectiveness in the treatments of certain diseases and ailments. Others are excellent insecticidal agents. However, accumulation of such compounds in the soil may negatively affect some metabolic and physiological processes of the same species or other plants through a phenomenon called allelopathy (Inderjit and Duke, 2003).

Allelopathy is commonly defined as direct or indirect effect of one plant on another. The compounds, collectively known as allelochemicals or bio-communicators are described as waste products of plant metabolic pathways which get into the atmosphere through root exudation, foliar leaching, volatilization and litter decomposition (Bhowmik and Inderjit, 2003). Unlike competition for growth resources, allelopathy is a form of chemical warfare among plant species competing for limited resources such as light, water and nutrients.

In places like Africa and Asia, investigations into the stimulatory and inhibitory effects of allelochemicals on different plant species have been reported (Inderjit and Duke, 2003). Recently, the inhibitory potentials of crop residues and straws in the management of non crop plants have motivated research interest on allelopathy (Fujii et al., 2003). Similarly, the allelopathic effects of secondary metabolites from various species of indigenous plants including those of medicinal values on the growth and yields of crops are also on the increase. Medicinal plants like any other vegetation are essential component of many traditional cropping systems that embrace management of several plants (Anaya, 1999). However, the extracts or decomposed residues of different herbal species adversely or favourably affect germination, growth and nutrient uptake of other plants or crop cultivars through the release of phytotoxic compounds into the soil (Maharjan et al., 2007).

In South Africa, previous studies conducted on related topic focused majorly on inhibitory effects of different tree species (*Tetrapleura tetraptera* and *Acacia mensii*) and an invasive weedy species (*Parthenium hysterophoruos*) on the germination and seedling development of crops and other vegetations (Amoo et al., 2008; Fatunbi et al., 2009). In spite of the ecological and agronomic importance of allelopathy in agroecosystems, scientific information regarding the negative influence of integration or sequential rotation of medicinal herbs with crops is limited in this part of the country. Therefore, there is the need for a systematic investigation and documentation of scientific evidence of the allelopathic effects of medicinal species growing in association with traditional crops on the same piece of land.

1.2 Rationale for the study

In South Africa, the high demand for herbal remedies has led to over-exploitation of many medicinal species. Thus, the fear of near-extinction of some valued wild indigenous plants has motivated research towards the development of alternative means of propagation (BAH, 2002). Tissue cultural techniques and /or integration of medicinal herbs into the traditional farming systems have been proposed (Lambert et al., 1997; Adebola and Afolayan, 2006). However, the detrimental effects of inclusion of such plants into the existing farming operation on the ecology, environmental degradation and conservation of genetic biodiversity require a better understanding (Anon, 2002).

In Asia, investigations into the allelopathic effects of crude extracts or residues of different medicinal plants on germination and seedling growth of both crops and non crop species have been conducted extensively. In Africa, there is dearth of information regarding the allelopathic effects of decomposing plant residue on germination, seedling growth and crop yield. This study therefore aims to investigate the allelopathic effects of the crude extracts and residues of *Arctotis arctotoides* L.f. O. Hoffm (an evergreen medicinal plant) on selected vegetable crops in Nkokombe Municipality, Eastern Cape Province of South Africa. Production and consumption of high quality vegetables is one of the government policies aimed to eradicate vitamin deficiencies among the rural communities (Reardon et al., 2003). Studies like this will help to know the stimulatory or inhibitory effects of the residues of medicinal species growing in association with traditional crops on the same field. The study will also validate the effects of treatments on the nutrient content of vegetable leaves in order to alleviate the problems of low vitamins intake by poor-resource farmers.

1.3 Objectives of the study

The broad objective of this study is to investigate the allelopathic effects of the root and shoot materials of *A. arctotoides* on the germination, growth, morphology, chlorophyll accumulation and nutrient uptake of some vegetables in the Nkokombe Municipality, Eastern Cape Province of South Africa. The specific objectives are to:

- examine the foliar ultra structures of *A. arctotoides* using Scanning Electron Microscope.
- carry out qualitative and quantitative phytochemical analysis of the root and shoot materials of *A. arctotoides*
- evaluate the inhibitory effects of the root and shoot aqueous extracts of *A. arctotoides* on the germination and seedling development of four vegetables in a laboratory bioassay
- assess the effects of the dried shoot residue of *A. arctotoides* on the growth, morphology and chlorophyll contents of cabbage and tomato in the greenhouse.
- determine the inhibitory effects of the dried shoot residue of *A. arctotoides* on the nutrients uptake, fruit yield and head weight of tomato and cabbage.

CHAPTER TWO

LITERATURE REVIEW

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Allelopathy: An Overview

Historically, “Allelopathy” was derived from the Greek words *allelon* "of each other" and *pathos* "to suffer" which literally translates to mutual suffering (Rizvi et al., 1992). Early research on allelopathy grew out of observations of poor regeneration of forest species, crop growth and yield reductions as well as occurrence of weed-free zones around some plants. In a historical overview, Willis (1985) pointed out that allelopathy is not a new concept. Contrary to the common problem observed during the Greek and Roman times on the so-called soil sickness attributed to perpetual decline in the yields of field crops, de Candolle (1832) suggested the involvement of plant-produced chemicals and their interactions in both natural and agroecosystems.

Reductions in the growth of crops planted following sorghum harvest according to Breazeale (1924) were attributed possibly to the release of some toxic compounds into the soil. Furthermore, the concept of allelopathy and the inclusion of harmful and beneficial effects of the phenomenon on neighbouring living organisms were developed by Molisch (1937). Although allelopathy has been variously described by different authors, the involvement of lower and higher plants as well as micro-organisms in the production of organic compounds has further broadened the definition (Cheema, 1988). During the World Congress on Allelopathy, the term was defined as: “Any process involving the production of secondary metabolites by both plants and microorganisms

including viruses, fungi and algae that influence the growth and development of biological systems positively or negatively (IAS, 1996).

The increasing awareness of the stimulatory or inhibitory effects of plant secondary metabolites on the growth of a particular species or other plants has led to series of unresolved arguments (Rice 1984). For these reasons different terminologies have been proposed based on the impact of organic substances released into the soil. For example, Putnam and Duke (1978) suggested that plants which release the chemicals should be identified as the 'donor' and the 'recipient' as the 'target'. Similarly, Whittaker and Feeny (1971) coined the term 'plant allelochemicals'. Allelochemicals according to these authors sometimes act as secondary metabolites; however, Berenbaum (1995) suggested that the two terms should not be used synonymously. Other scientific scholars (Khalid et al., 2002; Kobayashi, 2004) further described allelochemicals as allelopathic agents, bio-communicators, organic compounds, secondary metabolites, phytochemicals and phytotoxins.

In other reports, Reese (1979) defined allelochemicals as 'non-nutritional substances produced by an organism that affects the growth, health and behavior or population biology of other species. Blum et al. (1999) later suggested the criteria for proof of allelopathy which stipulated that the organic complexes (allelochemicals) produced and released by the donor plant or its debris must be in sufficient quantities capable of inhibiting the growth or influence some metabolic functions in the target organisms. For many decades, the effects of allelopathy on crop growth and yields have been evaluated only by botanists using crude aqueous extracts of plants (Weston, 1996). Recently, research into the isolation, purification and identification of specific natural products has

involved the joint contributions of organic chemists, biologists, soil scientists, and ecologists (Einhellig, 1995).

2.2 Allelochemicals

Natural products are secondary metabolites of plants, fungi or bacteria origin (Duke and Lydon, 1987). Although, earlier studies have illustrated that the organic compounds from plants are always in minute quantity, this may not necessarily be so because their production has been linked with factors such as biotic and abiotic stresses (Vaughn and Spencer, 1993). Similarly, Whittaker and Feeny (1971) also suggested that allelochemicals are not always present in all living organisms but a host number of these constituents are synthesized intermittently. Based on this assumption, numerous approaches have been put forward for proof of allelochemicals in the environment. According to Koch's postulates, the following steps were suggested: (i) demonstrate interference, describe symptoms and compare the effect using suitable controls; (ii) isolate, identify and characterize the chemical agent in suitable bioassays; (iii) reproduce the interference using a natural or synthetic agent to the biological system at naturally occurring concentrations (iv) quantify the amount of allelochemicals released from the donor plant and that absorbed by the target.

While the above outlined protocols have been proved difficult and unreasonable by some researchers, Weidenhamer (1996) asserted that the procedures are useful guides for a more accurate experimental design. However, a similar but less quantitative set of

components enlisted by Inderjit and Weston (2003) include: (i) ecological- demonstrating the existence of interference in nature; (ii) chemical-involving isolation, identification and characterization of allelochemicals; (iii) physiological-identification of the interference mechanism at the biochemical, physiological, cellular and molecular levels. These recommendations have led to series of investigations into the chemical composition of numerous natural products with different chemical structures (Razavi, 2011).

These authors further asserted that the active compounds must be isolated in sufficient quantity, adequate for identification and characterization in bioassays. In these bioassays, fractions from extracts or leachates should be screened on the germination of different seed species using different methods (Macías et al., 1998). For an ecologically relevant data, many scientists have proposed that the choice of extraction solvents and the methods should be given special consideration. Among the various methods adopted, the use of sealed culture-dish, re-circulating hydroponic culture system, GC/MS, HPLC and chromatography have all demonstrated the possibility of allelopathy in both hydrophilic (phenolics) and hydrophobic (terpenoids) compounds (Scrivanti et al., 2003; Sodaeizadeh et al., 2009).

A number of reviews on allelopathy confirmed that majority of the alleged phytochemicals represent a multitude of compounds from simple hydrocarbons and aliphatic acids to complex poly-cyclic structures (Putnam and Tang, 1986). These compounds were further divided into major chemical groups such as water-soluble organic acids, simple phenolics, benzoic acid and derivatives, lactones, flavonoids, tannins, terpenoids, monoterpenes, steroids, alkaloids and coumarins as well as amino acids and polypeptides (Macías et al., 1998). Among the different compounds, phenolic

acids, flavonoids, alkaloids, coumarins and terpenoids have been reported as the most frequently occurring phytochemicals present in many plant parts (Kovácik et al., 2007).

Mode of action of allelochemicals in plants

Determination of the mechanism of action of natural compounds has been described as challenging due to the large number of potential molecular target sites among the different targets (Dayan et al., 2000). In spite of the agronomic importance of allelopathy, relatively little is known about the mode of action of many allelochemicals or the adaptive strategies developed by several targets (Bais et al., 2006). Presently, there have been no established standard methods to study the mode of action of allelochemicals (Einhellig, 1985). Several approaches suitable or those that best address the needs of the investigators have been developed by different research teams. Sometimes, the team often relies on methodologies with which they are most comfortable. Selection of a putative site of action where numerous biologically active molecules can be tested with the hope of obtaining a "hit" for that specific molecular target is one approach.

For example, Dos Santos et al. (2004) reported that multiple physiological and anatomical responses usually observed in many targets due to the application of ferulic acid to the root are water utilization, foliar expansion and root elongation. Ferulic acid induces lipid peroxidation, and disrupts certain enzymatic activity thereby leading to membrane permeability and eventual blockage of plant nutrient uptake (Weir et al., 2004). Meanwhile, the allelochemicals may be esterified with the cell wall

polysaccharides, incorporated into the lignin structure or develop bridges that connect lignin with the wall of polysaccharides, thus making the cell wall rigid and restricting cell growth (Sánchez et al., 1996; Lam et al., 2001).

2.3 Sources of allelochemicals in the environment

Microbial source

Several classes of allelochemicals from bacteria and fungi have been isolated and identified. Currently, many of the products isolated from micro-organisms are being considered as new lead structures for the preparation of different analogues. For example, Ma et al. (1997) reported the discovery of phenolic acids, aldehydes, alcohol, ketones, and nitrogen-containing phytochemicals from fungi and bacteria. Similarly, bialaphos and phosphonthricin from a non phytopathogenic bacteria strain- *Streptomyces* have been commercialized and marketed under different trade names (Dayan et al., 1999). Although, most phytotoxins isolated from microbes have been found with numerous advantages over other biocontrol agents. However, problems associated with their storage, application and half-life favour plant-produced allelochemicals over those of microbes.

Plant source

Generally, green plants produce a large number of compounds whose specific functions are largely unknown (Duke and Lydon, 1987). Majority of these constituents however, have been vindicated as chemical warfare agents of insects, pathogenic organisms, and

competing plant species (Asawalam, 2006). Two important metabolic pathways noted for the synthesis of most allelochemicals in plants namely shikimate / phenylpropanoid and the terpenoids and their derivatives, originate from glyceraldehyde-3-phosphate (pyruvate) and cytoplasmic acetate (mevalonate) (Inderjit et al., 1999). The phenyl-propanoid pathway is the major source of phenolic compounds and a wide range of secondary products in plants including: ferulic, cinnamic and vanilic acids. These compounds together with several other allelopathic mono-, sesqui- and diterpenes are abundant in the soil (McConkey et al., 2000).

Studies on the production of phytochemicals of plant origin have been a subject of debate among researchers. While some authors asserted that organic compounds can be synthesized by living plants, others investigators assumed that chemical compounds can be produced passively during the decomposition processes of the plant residues (Blum et al., 1999). In line with the above findings, Sharma et al. (2003) pointed out that at the spatial level, the production and storage potential of plant secretory structures have a direct bearing with the developmental dynamics of the donor plants. Shanker et al. (1999) established that as glandular trichommes over mature, subcellular integrity deteriorates and gradually leaves the secretory organ empty. It has been well documented that numerous classes of allelochemicals are present in virtually all plant tissues and organs. Aldrich (1984) indicated the occurrence of higher concentrations of such compounds in the leaves, stem or roots rather than in the fruit or flowers.

Worldwide, scientific evidence has corroborated the occurrence of enormous quantities of allelochemicals in the leaves of the different plant species, genera and families

(Pandey et al., 1993; Taiwo and Makinde, 2005). However, the four main mechanisms through which organic compounds get into the environment are root exudation, leaching, volatilization from the aerial surface and residue decomposition (Putnam, 1985).

2.4 Mechanism of release of allelochemicals

Root exudation

The root system, otherwise known as 'hidden half' of a plant is an important component necessary for the acquisition of mineral nutrients and other solutes within the soil medium as well as anchoring the plant firmly to the soil. The actively growing root system, sometimes, may be expansive for the production and release of different classes of organic substances (root exudates) into the soil (Hopkins et al., 1998; Uren, 2000). Root exudates have been classified as low and high molecular weight compounds secreted during the process of secondary metabolism (Rice, 1979). Low-molecular organic compounds include acetic, ascorbic, benzoic, ferulic, malic and phenolic acids. Others such as flavonoids, fatty acids, nucleotides, tannins, steroids, terpenoids, alkaloids and vitamins are regarded as high molecular weight allelochemicals (Leather and Einhellig, 1985).

On the basis of molecular weight, root exudate products get into the rhizosphere from the living root membrane interface through three major processes namely; diffusion, ion channel and vesicle transport. The quantity, chemical composition, concentration and localization of root exudates have been attributed to plant age and species (Inderjit and Duke, 2003). Other factors such as soil compaction, drought, and low nutrient status have also been implicated on root exudate production. For example, production of phenolic

compounds by certain tree and leguminous plant species has been linked with phosphorus deficiency in the soil (Neumann et al., 1998). The efficacy or biological activities of the exudate products may be inactivated or modified by microbial decomposition or immobilization by soil particles (Cheng, 1992).

Leaching

Large amount of organic and inorganic metabolites are released into the soil through leaching. In spite of the numerous studies, there has been no adequate proof on the mechanism involved in the leaching processes. However, Tukey (1969) reported that injected metabolites into the plant tissues could be leached and collected. Metabolically important materials that could be leached from the above ground plant parts include; fructose, glucose, sucrose and polysaccharides. Allelochemicals such as organic acids and other growth regulators, nutrients and amino acids have also been included in the leached products. Although, many of these metabolites exhibit both inhibitory and stimulatory effects on different targets under certain circumstances, however, the nature of these two effects depends on the concentration and the physiological activities of the compounds involved. Factors such as physiological age of the plant and molecular weight of leached products are greatly influenced by leaf characteristics and leaf surface area, plant nutrition, rainfall intensity and volume as well as light intensity and temperature (Kobayashi, 2004).

Volatilization

Volatile chemicals that mediate interaction between different species benefit both the emitting and the receiving organisms. Volatile compounds such as synomones, allomones and kairomones are the major components of many terpenes. For example, monoterpenes, sesquiterpenes and alpha-pinenes are the largest group of organic constituents of essential oils responsible for biochemical interactions among plants (Fischer 1986; Einhellig and Leather 1988). It has been demonstrated that monoterpene vapours inhibit seed germination as well as causing anatomical and physiological changes in the seedlings of many plants (Koitabashi et al., 1997; Dudai et al., 2000). Sesquiterpenoids on the other hand, are C-15 volatile derivatives less commonly found in the essential oils fractions because of their higher molecular weight. Alpha-pinene is reported to be present in several aromatic plant species including the forest trees (Llusià and Peñuelas, 2000).

Residue decomposition

Studies on soil quality and productivity have shown that physical conditions of the soil surface are determined by the presence of organic matter (Sharma and Bhushan, 2001). Apart from nutrient recycling, incorporation of plant residues into the soil improves soil structure and physical characteristics. However, it has been well documented that the residues of plants under stressful environmental conditions are prone to production of higher quantities of allelochemicals (Einhellig, 1995). Directly or indirectly, the residues of certain plant species or crop cultivars release organic compounds that adversely affect the metabolic processes and growth of other plants (Jäderlund et al., 1996). Utilization of

reserve mineral elements as energy source by micro-organisms creates abiotic stresses for the plant by interfering with the uptake of certain mineral nutrients or their mineralization. The severity of the problem can be more if the residue is from a donor plant previously under nutritional deficiency.

The increase in relative phytotoxicity of aqueous extracts over time and its decline has been a subject of debate (Mersie and Singh, 1987). However, increase in phytotoxicity of up to 60 days of decomposition before decline may indicate that allelopathic effect of plants could sometimes increase over weeks or months of decomposition prior to declining (Wilson, 1981).

2.5 The role of allelopathy in the environment

Effects of allelopathy on soil and microorganisms

Mineralization and nitrification processes are the major limiting problems associated with soil nutrients (nitrogen and phosphorus) availability in many areas of the world (Zhang and Yu, 1989; Anaya, 1999). Allelochemicals may influence these two processes through their effects on soil symbiotic microbes. For example, Hoagland and Williams (1985) confirmed that as the root system develops in the soil, the soil microenvironment is altered; the organic and inorganic compounds exuded from roots either stimulate or inhibit the growth of the micro-organisms. In a related study, Taiwo and Makinde (2005) reported that microorganisms can modify the configuration of allelochemicals thereby leading to formation of new compounds which could be stimulatory or inhibitory to the growth of some fungi and bacteria in the soil. At varying concentrations, the stimulatory

and inhibitory effects of the different aqueous extracts of several plant species have been observed on numerous targets (Pue et al., 1995).

Effects of allelopathy on forest and orchard plantation

The problems associated with regeneration and replanting of forest and fruit trees in orchards have been elucidated (Mallik, 1998). Several reports have indicated the inhibitory effects of allelochemicals on the growth of forest and understory species (Lovett and Ryuntyu, 1992). Hassan et al. (1989) corroborated early study that soil previously grown with citrus contained compounds that adversely affect succeeding citrus seedlings. Similarly, Amoo et al. (2008) reported the effect of the leaf extracts of *Tetrapleura tetraptera* (a multipurpose tree) on selected agricultural crops. In like manner, Fatunbi et al. (2009) attributed the occurrence of dense population of *Acacia mearsii* to the hydrological imbalance of water bodies while Duryea et al. (1999) reported the effect of aqueous extracts of eucalyptus and mulches of pine-straw on the germination of lettuce seeds.

Effects of allelopathy on crop plants

Traditionally, several crop combinations including cereals, legumes and vegetables are found on the same field, same time or in rotation for self-sufficiency (Chon et al., 2005). In addition to the various environmental stresses (diseases, insect attack and competition) facing the different crop species, allelopathy has also been implicated in crop growth failure and yield reductions (Young, 1998). Unlike competition for resources, allelopathy is an obvious, more subtle interference involving the release of chemicals into the environment (Inderjit and Duke, 2003). The mechanisms through which several classes

of allelochemicals are released into the soil by donor plants have been well documented (Cheng, 1992).

Positively or negatively, the chemicals affect germination and seedling development of the recipients directly or indirectly through microbial degradation (White et al., 1989). However, the beneficial role of allelopathy has been observed in various cropping systems such as mixed cropping, multiple cropping, cover cropping, crop rotation, minimum and no-tillage on the field (Leather, 1987). Although, little attention has been given to the adverse effects of allelochemicals on crop growth and yields, reports from laboratory bioassays have confirmed the inhibition or delay in the germination of certain crop seeds while no significant effects were observed on the yields of these species (Lin et al., 2006). Worldwide, significant progress has been made by numerous research groups towards selection of allelopathic crop varieties using their aqueous extract, mulches or residue (Chon et al., 2005).

Moreover, recent reviews and scientific reports on the position of allelopathy in agroecosystems have evaluated extensively the effects of: crops on non crop suppression, crops residues on succeeding crops, weed residues on crop yield, weed residues on weed interactions, weed seeds on crop growth (Weston and Duke, 2003; Nazir et al., 2007). Apart from agricultural crops, screening of other non crop plants such as multipurpose and fruit trees as well as medicinal shrub species for their allelopathic activities have also been documented (Khan et al., 2008; Javaid et al., 2009).

2.6 Screening of plants for allelopathic properties

Screening of plants for allelopathic activity has been reported to be similar to those for the discovery of new lead compounds in the pharmaceutical industry (Vyvyan, 2002).

Fujii et al. (2003) emphasized that the preliminary criterion for evaluating allelopathic potentials of plants should consider factors such as the growth habit, population density and dominance of the bioassay species in the ecosystems. Several methods have been suggested by different workers for evaluating the allelopathic properties of plants. However, the first strategic approach involves laboratory bioassay which is usually followed by greenhouse or field studies (Dayan et al., 2000).

Practices such as grinding or chopping of plant materials, use of organic solvents for the extraction and autoclaving of assay media have been controversial (Khan et al., 2009). These authors believed that most phytotoxic compounds are discharged into the soil by rain and soil-water under natural condition. In contrast, Anaya (1999) disputed the statement and suggested that other volatile compounds such as monoterpenoids and sesquiterpenes lactones might get into the soil through volatilization, root exudation and residue decomposition. However, two important standardized protocols for proof of allelopathic interactions in both laboratory and greenhouse studies put forward are; selection of biological assays (bioassays) and the target species.

Laboratory* and *greenhouse bioassays

Laboratory and greenhouse bioassays are important procedure for the establishment of allelopathic potentials of pure compounds, extract or decomposed residues of the suspected allelopathic plant (Inderjit and Dakshini, 1995). Earlier findings have shown that these two preliminary bioassays are useful techniques that distinguish between competitive interference and allelopathy under field conditions (Xuan et al., 2005). Under natural environment, field trials are important in the estimation of allelochemicals

however, seed germination and seedling growth are the most widely indices used for evaluating the impacts of allelopathic interference. In laboratory assays, seeds of selected targets are usually kept on growth media such as filter paper, agar in a Petri dish or tissue culture wells. Usually, the media is moistened with a known volume of the extract or weight of the residue (Singh et al., 2003a; Chaniago et al., 2008). More importantly, seed size, number per Petri dish and the volume of the extract to be applied are other factors being considered.

Extraction solvents and methods

Water is a universal solvent most widely used for extraction in nature. Processes such as root exudation, leaching and residue decomposition require water for microbial growth and dissolution of organic compounds. Amongst the numerous solvents being adopted for the extraction of natural products from plants, distilled water remains the most popular and regular solvent used in most bioassays. The use of organic solvents as extraction agent has been a subject of debate by many researchers (Baruah et al., 1994). For example, Inderjit and Dakshini (1995) suggested that several qualitative and quantitative differences exist between compounds extracted with both aqueous and organic solvents. However, where necessary, acetone and dimethyl sulfoxide (DMSO) have been universally accepted as being appropriate for the transfer of compounds with sparingly water solubility (Dayan et al., 2000). In line with this suggestion, the use of non-polar solvents in the transfer of volatile compounds (essential oil constituents) to assay wells has been adopted by other authors (De Martino et al., 2010).

In spite of the different methods of extraction being demonstrated on the allelopathic possibilities of several bioassay species, several hypotheses are still unresolved among

researchers. This has invariably led to the establishment of routine and specific methodologies involving the use of crude extracts, leachates from live or decaying plant materials (Koyabashi, 2002; Belz et al., 2007). Among others, various methods of extractions have been suggested by numerous scientists and these include sandwich (Fujii et al., 2004), cluster analysis using HPLC (Mungole et al., 2010) and agar medium selection (Fujii et al., 1992). Other scholars (Navarez and Olofsdotter, 1996) also reported relay seeding technique and hydroponic culture assessment.

Concentration-dependent* or *Dose-response bioassay

Bioassay strategies based on concentration or dose dependent phytotoxicity are valuable indices of species sensitivity and tolerance to different allelopathic agents (Weidenhamer, 1996). The initial dose-response concentrations ranging between 10^{-4} and 10^{-9} M, with as little as a milligram of the plant material has been suggested (Dayan et al., 2000). Similarly, concentration-dependent relationship has been characterized by both stimulatory and inhibitory reactions at lower and higher doses respectively (Calabrese et al., 2007). This relationship has been observed with several classes of compounds isolated from crude plant extracts and root exudates of wheat (*Triticum aestivum* L.) (Belz et al., 2007).

Field experiments

In allelopathic research, field experimentation involves the exploration of the whole or different parts of allelopathic crops or other plant species as biological agent of weed control (Wu et al., 1999; Fujii et al., 2004). In the past, the role of factors affecting

phytotoxicity of many allelochemicals in the soil has received little attention. Conversely, Inderjit et al. (2001) reported that most allelopathic bioassays showed slight similarity in comparison with the observed effects under natural conditions. For this reason, several methods of residue applications have been proposed in the evaluation of the different allelopathic plant parts as control agent of natural vegetation (Terzi, 2008). Mulching with straw is a common practice in most agro based cropping systems of the developing world (Fujii et al., 2004). The use of fresh or dry crop or plant residues either as surface application, soil incorporated or rotational sequence in the field has been adopted by different authors (Pandey et al., 1993; Fatunbi et al., 2009). Based on these management practices, Batish et al. (2007) confirmed that most of the applied residue treatments show comparable results with those observed in herbicide treated plots.

Plant Organs* and *Tissues

The production of a wide range of organic substances by plants has been well documented (Viles and Reese, 1996). Other authors (Parvez et al., 2004) reported that several classes of secondary metabolites are stored in the different tissues and organs of the plants. However, Parekh and Chanda (2001) stated that generally, the aerial surfaces of many plants contain higher percentage of secondary metabolites than the other parts.

Target species

Selection of target species is an important aspect of allelopathic research considered for the accuracy in bioassay studies (Daizy et al., 2006). Generally, plant species vary in their susceptibility to allelochemicals particularly during the early stages of development

(Inderjit and Olofsdotter 1998). A wide range of sensitive targets have been studied under laboratory / greenhouse techniques and field conditions to evaluate allelopathy (Dakshini et al., 1999). Usually, seed species from both mono and dicotyledonous crops / plants are recommended while screening for allelopathic activities. This is with a view to determining the potential sensitivity of some targets for the development and breeding of crops with a competitive advantage over other plant species (Rice, 1984; Roth et al., 2000).

A considerable amount of research has been carried out to quantify the use of commercially available crop seeds. The adoption of this protocol eliminates the uncertainty that are usually associated with the use of genetically and more diverse weed seeds. It also ensures the development of standard targets for bioassay studies (Macias et al., 2000). Based on the set standards, the seeds of crops such as *Brassica oleraceae* (Cabbage), *Lactuca sativa L.* (Lettuce), *Lycopersicon esculentum L.* (tomatoes), *Raphanus sativus L.* (radishes) and *Oryza sativa* (rice) have been utilized by different authors in most allelopathic studies (Chung et al., 2003). Usually, the choice and selection of any of these seed species is attributed mainly to their sensitivity to allelopathic agents in addition to availability in the open markets.

2.7 Allelopathic plants

Allelopathic crop plants

The increased awareness motivated by the allelopathic interference among different crop plants grown in rotation or mixed cropping has led to the exploitation of this potential in agroecosystems (Hedge and Miller, 1990; Javaid et al., 2006). For example, germination

inhibition and growth retardation caused by aqueous extracts and residues of lettuce (*Lactuca sativa* L.), wheat straw (*Triticum aestivum* L.) and alfalfa (*Medicago sativa* L.) on other plants have been utilized in weed control and management (Chon et al., 2005). A number of review articles and scientific reports have evaluated allelopathy with respect to breeding of crops with competitive advantage over other vegetation. Since the major goal of allelopathy has been alternative weed control and management strategy using crop or plant residues, a number of reviewed and research reports have investigated breeding of crops with competitive advantage over other vegetation. Thus the effects of crops on non crop plants, crop residues on succeeding crops, weed residues on crop yield and weed seeds on crop growth have been evaluated (Tripathi et al., 1998).

The occurrence of several classes of organic compounds in both aqueous and organic solvent extracts of many plant and crop species have been well documented (Ashrafi et al., 2008). Allelochemicals such as phenolic (coumaric, ferulic, and cinnamic) acids and terpenoids derivatives (limonene, α-pinene, and 1-8 cineole) have all been reported as the most abundant naturally occurring organic substances ubiquitous in the decomposing residues and root exudates of several crops (Mandava, 1984). The compounds, singly or collectively cause metabolic disorders such as root lignifications, electrolyte leakages, lipid peroxidation and membrane deterioration of the recipient targets (Dos Santos et al., 2004). Apart from agricultural crops, certain tropical forest and fruit trees particularly those belonging to the different families have also been implicated in allelopathic interactions (Amoo et al., 2008). More recently, the involvement of bioactive constituents of medicinal importance in both stimulatory and inhibitory effects on other plants has

further motivated research into the allelopathic potentials of the different herbal species on crop growth and yield reductions (Lin et al., 2006).

Allelopathy in medicinal plants

Over the last few decades, the biological and pharmacological potentials of organic substances from many indigenous plants are well understood. For instance, phenolic compounds have been associated with antimicrobial (Narayana et al., 1999), anti-inflammatory (Castillo et al., 1989), antiviral, and cytotoxic activities (Chhabra et al., 1984). However, the bioactive constituents conferring these properties on many herbal species have also been implicated in allelopathy (Nazir et al., 2007). A number of review and research articles have indicated that phenolic acids are the most commonly occurring natural products noted for allelopathic activities (Singh et al., 2003b). Mungole et al. (2010) have also included alkaloids, coumarin, flavonoids, saponnins and volatile constituents of the essential oils as being allelopathic agents.

Generally, the presence of different phytochemicals in the crude plant extracts has been linked to the detrimental effects of leachates, root exudates or decomposing residues of such plants on the other vegetation or succeeding crops (Chung et al., 2005). Phytochemical analyses of several species of medicinal plants and allelopathic activities of the crude chemical compounds on crops and plants have yielded positive results (Fujii et al., 2004). Of the different plant families studied, Viles and Reese (1996) indicated that members of the *Asteraceae* family have great potential for inhibitory activities. Thus, the

increased interest in the isolation and identification of the chemical compositions of organic products associated with biological activities with particular emphasis on germination, growth and yield of crops has stimulated research on plants having both medicinal and allelopathic properties (Hegazy and Farrag, 2007). Although the inclusion of medicinal species as component crops for weed control and for commercial purposes might be justified. However, the negative impact of organic substances released into the environment by the species on the growth and yields of economic crops requires a better understanding (Lambert et al., 1997).

2.8 Allelopathy in South Africa

South Africa is one of the richest floristic regions of the world, well known for the different understory plant biomass and medicinal species. The Eastern Cape Province is one of the Provinces endowed with different plant species valuable for health care needs of the larger populations and fodder for the livestock (Van Wyk et al., 1997; Masika and Afolayan, 2003). Like many other developing nations, where several classes of plant secondary metabolites have been implicated in allelopathy, similar observations have also been well documented in South Africa (Fatunbi et al., 2009). Worldwide, utilization of the allelopathic characteristics of both plants and crop cultivars for the control or management of other vegetation in crop based agroecosystems is common. However, very little is known on the allelopathic interactions of medicinal species growing in association with traditional crops in Nkokombe Municipality, Eastern Cape Province of South Africa.

CHAPTER THREE

PLANT USED IN THIS STUDY

CHAPTER THREE

3.0 PLANT USED IN THIS STUDY

3.1 *Arctotis arctotoides* (L.f.) O. Hoffm.

Plants in the family *Asteraceae* produce wide varieties of biologically active constituents. The genus *Arctotis* belongs to the family and is one of the angiosperms endemic in southern Africa, having more than 50 species in the coastal districts and summer rainfall areas (Funk et al., 2005). Of particular interest is the species *Arctotis arctotoides* which is a member of the genus. It is a fast growing, evergreen perennial groundcover with light green foliage and butter-yellow flowers (Figure 3.1). The leaf and stem surfaces of the plant are usually covered with white hairs which are believed to be special ultra structures (Pooley, 1998). *Arctotis arctotoides* is widely distributed in disturbed habitats such as rain fed or irrigated lawns and cultivated fields. At maturity, the plant grows up to 60 to 65 cm in height and its leaves could be as long as 10 to 15 cm thereby forming a dense and patchy strand around other vegetations.

The plant dominates grasses and severely devastates crop plants particularly, vegetables on farmers' fields and in home gardens around the University of Fort Hare, in the Nkokombe Municipality, Eastern Cape of South Africa. *Arctotis arctotoides* was selected for this study because of its observed dominant growth habit and majorly due to the occurrence of different classes of phytochemicals in its root and shoot. For example, comprehensive GC-MS analyses of the two plant parts have revealed mono and sesquiterpene derivatives as the major volatile constituents of their essential oils (Oyedeji et al., 2005). Similarly, Sultana and Afolayan (2007) isolated a number of novel

compounds including: dehydrocostuslactone, nepetin, zaluzanin and pedalitin from the shoot of the plant.

Figure 3.1: *Arctotis arctotoides* (L.f.) O.Hoffm.

Economically, *A. arctotoides* is well known in the Eastern Cape Province of South Africa among the Xhosa speaking people for the treatment of diverse diseases. Topically, the juice from *A. arctotoides* leaves is also useful in wound healing. Extracts from the root and the shoot of the plant have been shown to be effective as antimicrobial and antifungal agents (Afolayan, 2003). However, many of the compounds isolated from the plant have been implicated in allelopathic activities against other crops (Fischer, 1986; Macías et al., 2000).

3.2. Vegetable crops used for this study

Inadequate intake of vegetables in diets has been associated with high rate of vitamin deficiency in developing countries (Flyman and Afolayan, 2006). Generally, the diet of resource-poor populations tend to be low in some micronutrients due to consumption of predominantly starchy staples with or without animal products and few vegetables and fruits (Faber and Van Jaarsveld, 2007). As a means to eradication of poverty, hunger and malnutrition, intensive vegetable production is on the increase in many of the affected nations through government policies (Reardon et al**.,** 2003). Home gardening has been shown as an important means of improving the intake of vegetables and fruits (Berti et al., 2004). In the Eastern Cape Province of South Africa, vegetables such as potato, spinach, beet root, cabbage, carrot, tomato and cucumber are common in many home gardens and semi-subsistence farming operations. Hence the selection of cabbage (*Brassica oleraceae*), carrot (*Daucus carota* L), tomato (*Lycopersicon esculentum* Mill) and spinach (*Spinacia oleraceae*) used in this study. The vegetables are short season and widely cultivated by the local people around the University of Fort Hare where *A. arctotoides* usually predominates.

Cabbage

Cabbage (*Brassica oleracea*) belongs to the family *Cruciferae*. It is a dicotyledonous biennial crop but is usually grown as annual (Figure 3.2.1). Many varieties are gaining popularity as components of prepackaged salad mixes. There are many variations amongst cabbage types, ranging in colours from green to purple; in leaf character from

smooth to savoy, in head shape from flat to pointed and in maturity from early to late maturing.

A B

Figure 3.2.1 : Cabbage (A) head and (B) seeds

The green and round headed types are the most common (Phillips and Rix, 1993). Culturally, the vegetable thrives best during the cool and moist weather. Sufficient amount of water and good drainage are necessary throughout the growing period of the vegetable particularly during head formation until harvest (Askew, 1999). The maturity period of most cabbage cultivars is between 80 to 100 days and according to Hadfield (1995), it could be cultivated throughout the year in most parts of South Africa depending on the soil types and its optimum pH.

Carrot

Carrot (*Carrot daucus*) is a biennial crop and member of the family *Apiaceae*. Other members of the family include celery, celeriac, coriander, fennel, parsnip and parsley. In South Africa, carrot is one of the top 10 vegetables cultivated on an area basis (Joubert et al., 1980). The vegetable grows vegetatively during the first season and produces seed in the second (Figure 3.2.2).

A B

Figure 3.2.2: Carrot (A) Fruits and (B) Seeds

For edible root production, the plant is grown as an annual crop. Nutritionally, carrot is rich in carotene **(**Michaela et al., 2000) and it is consumed either fresh or cooked. Large quantities of the vegetable are also processed either alone or in mixtures with other vegetables by canning, freezing or dehydration.

Spinach

Spinach (*Spinacia oleracea L.*) also known as chard, leaf beet or Swiss chard is produced mainly for its large crispy edible leaves which are cooked as green. (Figure 3.2.3) The vegetable grows best in summer but may survive over winter frost in temperate regions. Comparatively spinach is well suited to climates with an optimum temperature that ranges between 18 and 20^{o}C. Continued exposure to temperatures less than 5^{o}C induces seed production (bolting). Spinach grown during summer tends to develop small leaves which are of inferior quality. However, different methods of propagations have been investigated for selection of suitable cultivars. Among others, in vitro plant regeneration, soilless culture systems and hydroponic nutrient solution have shown increases in both the yield and quality of the vegetable.

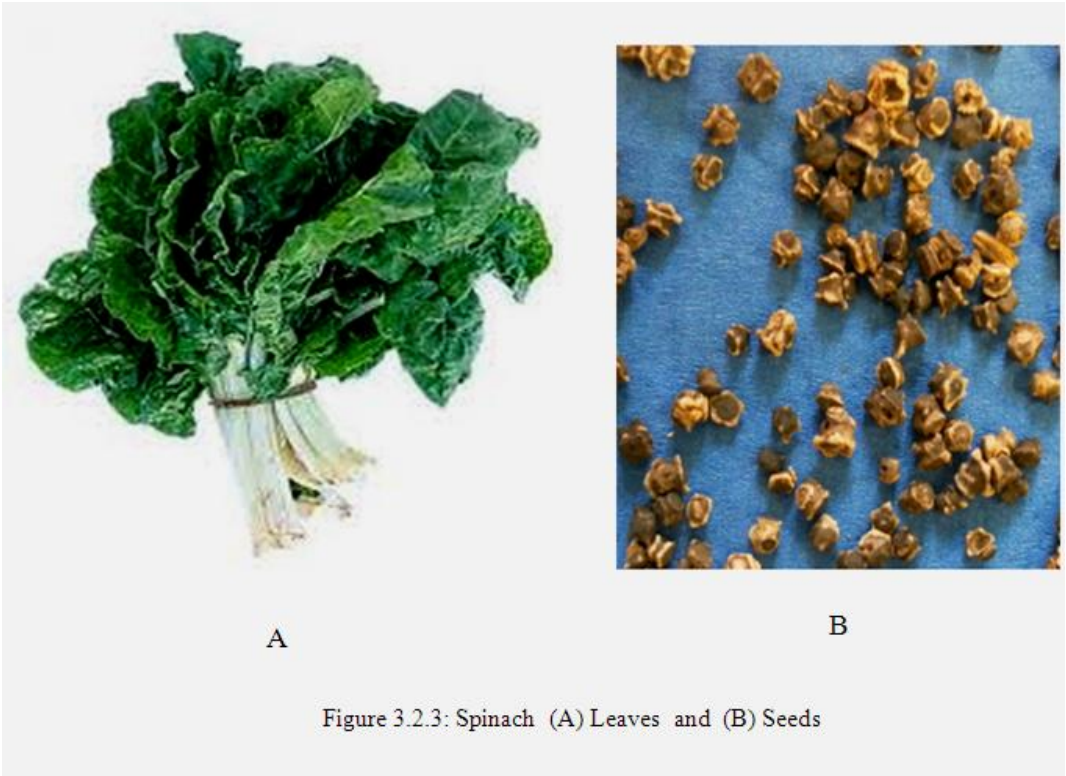

Figure 3.2.3: Spinach (A) Leaves and (B) Seeds

Tomato

Tomato (*Lycopersicon esculentum* L.) is one of the most popularly consumed vegetables in many parts of the world and it ranks third in terms of production after potato (*Solanum tuberosum* L.) and sweet potato (*Ipomoea batata* L.) (Figure 3.2.4). The crop is highly valued but sensitive to variations in the day / night temperatures in addition to considerable amount of inputs and good management requirements. Moderately cool night and day temperatures required for normal growth are between 15-20^{o}C and 21-28^{o}C respectively. In many Provinces of South Africa, especially Kwazulu-Natal, large scale production of tomato is under hydroponic culture and irrigation systems. The rural communities around the University of Forte Hare cultivate tomato mostly as one of the home garden and subsistence farming vegetables in association with other non crop species to cater for both nutritional needs and herbal remedies of the larger populace.

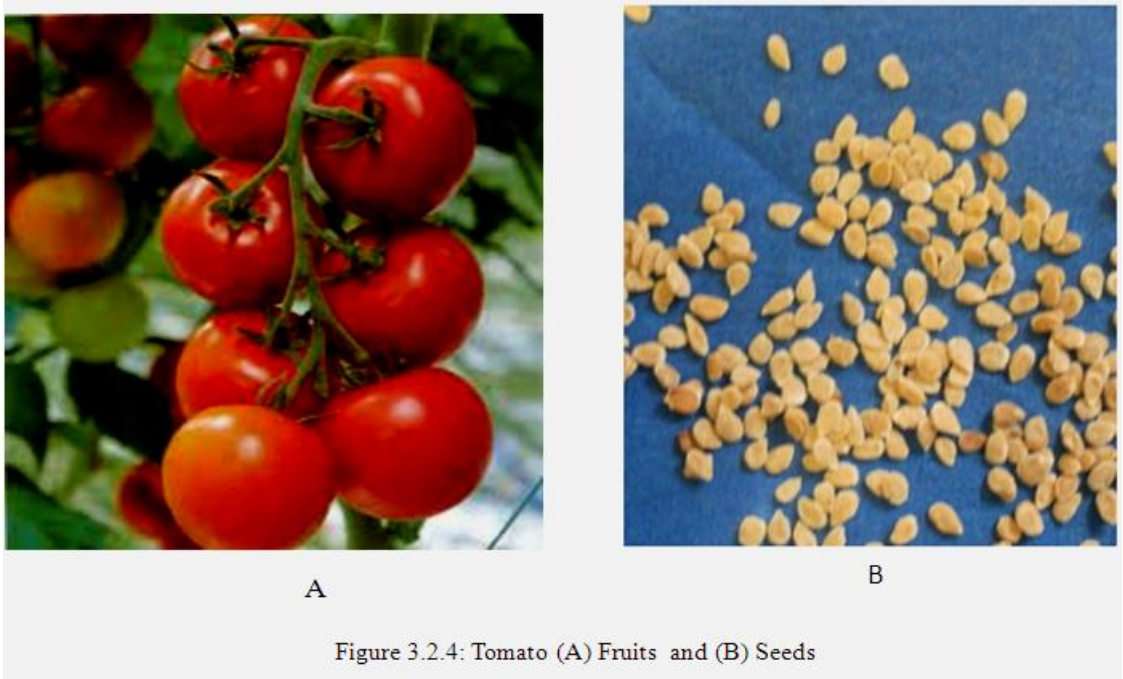

Figure 3.2.4: Tomato (A) Fruits and (B) Seeds

CHAPTER FOUR

MATERIALS AND METHODS

CHAPTER FOUR

4.0 MATERIALS AND METHODS

4.1 Experiment 1: The foliar micro morphology of *Arctotis arctotoides*

The whole plant of *A. arctotoides* was harvested during its vegetative stage from a natural population around the University of Fort Hare (UFH) Research Farm, Alice campus in August, 2008. Plant identification was done by Prof Grierson of the Department of Botany, UFH and a voucher specimen (Badmus Med. 2008/04) was kept in the herbarium. The foliar ultra structures of the leaf surfaces of the plant were examined using the general procedures for Scanning Electron Microscope (SEM) adopted by Vaishali et al. (2008). Freshly cut leaf samples were rinsed in distilled water and sectioned into about 4 - 6 mm segments before fixing in 0.05 M sodium cacodylate and rinsed again in 0.05 M cacodylate buffer (pH 7.5). The samples were further dehydrated in a graded series of ethanol (20 -100%) three times at 20 min per rinse. This was followed by critical point drying with liquid carbon-dioxide in Hitachi HCP-2 Critical Point Dryer. Each dried sample was mounted unto aluminum specimen stubs with double-sided carbon coated adhesive discs and sputter coated with gold-palladium (Eiko IB · 3 Ion Coater). Using JEOL (JSM-6390LV) SEM, operated at 10 – 15 kV acceleration voltage, the adaxial and abaxial surfaces of the leaf specimen were examined at varying magnifications. All the representative features examined were captured digitally using Microsoft image soft ware for windows. The energy dispersive X-ray spectroscopy-SEM,

involved both the fixing and dehydration procedures as in SEM, while a FEI QUANTA 200 Oxford EDX Analyzer was used for the analysis.

4.2 Experiment 2: Qualitative and quantitative phytochemical screening of the root and shoot residues of *A. arctotoides*

Plant materials obtained as described in the previous experiment were sectioned into root and shoot and then air-dried at room temperature for two weeks. The dried plant samples were later ground to a uniform texture and used for the respective analyses.

4.2.1 Qualitative determination of phytochemical constituents

Qualitative phytochemical analyses of the root and shoot ground materials of *A. arctotoides* were determined by adopting the procedures described by Harborne (1973); Trease and Evans (1989); Sofowora (1993); Awe and Shodipo (2001) and Edeoga et al. (2005).

Test for alkaloids

Each of the plant samples (0.2 g) was separately dissolved in 10 ml of methanol and filtered. The filtrate (2 ml) was treated with 1% HCl and 6 drops of dragendrorff's reagent (0.11 M potassium iodide and 0.6 M bismuth nitrate in 3.5 M acetic acid). The mixtures were kept on a water bath to steam for 30 min. Formation of turbidity or creamish precipitate indicated the presence of alkaloids.

Test for cardiac glycosides (Keller-kilian test)

Aqueous extract of the two plant parts (5 ml) was added to 2 ml of glacial acetic acid plus one drop of $FeCl_3$. The set up was underplayed with 1 ml of concentrated H_2SO_4. The appearance of violet and brownish rings below the interface followed by formation of a greenish ring in the acetic acid layer indicated the presence of cardiac glycosides.

Test for flavonoids

The plant material (0.2 g) was dissolved in 10 ml of ethanol and filtered. The filtrate (2 ml) was added to 1% concentrated hydrochloric acid plus magnesium ribbon. Formation of pink to reddish colouration signified the presence of flavonoids.

Test for phenolics

The ground materials (5 g of each) were extracted in 20 ml of distilled water. To 1 ml of the plant extract, one drop of 5% $FeCl_3$ (w/v) was added. Formation of greenish precipitate indicated the presence of phenolics.

Test for saponins

The powdered material (10 g) was boiled in 20 ml of distilled water in a water bath for 2 h and then filtered. The filtrate (10 ml) was diluted with 5 ml of distilled water and shaken vigorously for a stable persistent froth. Three drops of olive oil was added followed by vigorous shaking. The set up was observed after few minutes for the formation of emulsion, indicating the presence of saponins.

Test for steroids

The plant material (0.2 g) was extracted in 10 ml of chloroform and filtered. The filtrate (2 ml) was added to 2 ml of acetic anhydride and mixed with 2 ml of concentrated H_2SO_4. Formation of blue-green ring implied the presence of steroids.

Test for tannin

The powdered plant sample (0.5 g) was boiled in 20 ml of distilled water in a 50 ml beaker and filtered. The filtrate was treated with few drops of 0.1% $FeCl_3$ and observed for blue-black or brownish-green colour development that signified the presence of tannin.

Test for triterpenes

One ml of the root and shoot aqueous extracts was treated with five drops of acetic acid anhydride followed by a drop of concentrated H_2SO_4. The mixture was kept on a water bath for 60 min and neutralized by addition of NaOH. This was followed by addition of chloroform. A blue-green colour indicated the presence of triterpenes.

4.2.2 Quantitative determination of the phytochemical constituents

Determination of total phenol

Total phenolic content was determined using Folin Ciocalteau reagent. An aliquot of the extracts was mixed with 5 ml of the reagent (diluted with water 1:10 v/v) and 4 ml of Na_2CO_3 (75 g/l). The tubes were vortexed for 15 s and allowed to stand for 30 min at 40^o C for colour development. The absorbance was measured at 765 nm wavelength. Samples of the extract were evaluated at a final concentration of 0.1 mg/ml and total phenolic

content was expressed as mg/ml tannic acid equivalent using the calibration curve of the equation: y = 0.1216 x, R2 = 0.9365, where x = absorbance and y = tannic acid equivalent (mg/ml).

Determination of total alkaloids

Five g of the ground plant sample was weighed into 250 ml beaker, 200 ml of 10% acetic acid in ethanol were added and left for 4 h. The filtrate was concentrated on a water bath and reduced to one-quarter of the original volume then allowed to settle. Concentrated NH_4OH was added drop wise till precipitation was complete. The precipitate collected was again washed with dilute NH_4OH, filtered and dried. The dried residue was then weighed and expressed as alkaloid percentage.

Determination of total flavonoids

$AlCl_3$ ethanol solution (2%, 0.5 ml) was added to 0.5 ml of the extract solution and left for 60 min at room temperature. The absorbance was measured at 420 nm and total flavonoids contents were calculated as quercetin (mg/g) using calibration curve equation: y = 0.0255x, R^2 = 0.9812, where x was the absorbance and y was in quercetin equivalent (mg/g).

Determination of total tannin

The plant sample (0.5 g) was added into 50 ml of distilled water in 100 ml beaker and kept on orbital shaker for 60 min. The filtrate was made up to mark in a 50 ml volumetric flask out of which 5 ml was pipetted and mixed thoroughly with 2 ml of 0.1 M of $FeCl_3$ in 0.1 N HCl and 0.008 M potassium ferrocyanide all in a test tube. The absorbance of the solution was read at 120 nm within 10 min.

4.3 Experiment 3: Allelopathic activity of the root and shoot aqueous extracts of *A. arctotoides* on cabbage, carrot, tomato and spinach seeds

Actively growing plants population of *A. arctotoides* was harvested in November 2009 around the University of Fort Hare. The root and shoot samples of the plants were rinsed repeatedly under running tap water and then distilled water. Samples were air-dried for 3 days and later oven dried for 72 h at 65^{o}C. The dried plant materials were passed through a hammer mill attached to 2 mm sieve to obtain a fine powdery substance. The powdered root and shoot materials were used for experiment 3 whereas the shoot residue alone served as experimental unit for those of 4 and 5.

Preparation of aqueous extracts

The root and shoot aqueous extracts of *A. arctotoides* were prepared by suspending 50 g each of the powdered samples of the two plant parts in 2 L of distilled water at room temperature. The set up was kept on an orbital shaker (SOI Stuart Scientific Stone U. K) for 12 h and vacuum filtered through a Whatman (No. 1) filter paper. The dry matter yields (14.08 g) for the shoot and (15.19 g) for the root were transferred into a tightly sealed glass bottle and kept at 4^{o}C until needed.

Seed viability test

The seeds of cabbage (*Brassica oleracea* var. *vulgare* L. cv. Drumhead), carrot (*Carrot daucus* L. cv. Kuroda), spinach (*Spinacia oleracea* L. cv. Fordhook Giant) and tomato (*Lycopersicon esculentum Mill.* cv. Heinz HT G08004) were obtained from Hygrotech

Commercial Seed Centre, East London, South Africa. The viability of the vegetable seeds was tested by adopting early procedure of Kambizi et al. (2006). Twenty five seeds of cabbage, carrot, spinach and tomato were randomly arranged inside 9 cm Petri dishes containing adequate quantity of distilled water. After 24 h of soaking, the seeds were dissected into two equal halves. The seed samples were again soaked in 0.01% 2, 3, 5 tetraphenyl tetrazolium chloride (TTC) for 16 h at 25^{o}C in the dark and then viewed under the light microscope after rinsing in several changes of distilled water.

Seed treatment and preliminary germination trials

The four vegetable seeds were sterilized in 0.1% sodium hypochlorite for 60 s and then rinsed repeatedly under running tap followed by distilled water before air-drying using a 53µm sieve. Twenty five seeds each of the four vegetables were arranged in 9 cm Petri dish lined with two Whatman filter papers and moistened with various volumes of distilled water (1 to 10 ml) and then incubated for 14 days at 25 to 27°C to determine the optimum volume of water required for normal seed germination and growth.

Investigations into allelopathic effects of the root and shoot aqueous extracts of *A. arctotoides* on seed germination and seedling growth

Studies on seed germination and seedling development of cabbage, carrot, spinach and tomato were conducted by adding 6.5 ml of each extract at concentrations of 10, 8, 6, 4 and 2 mg/ml and distilled water (control) to 9 cm diameter Petri dish lined with two filter

papers. Four replicates of 25 uniform seeds of each vegetable were distributed evenly on the filter papers and the dish was covered with its lid and sealed with parafilm before being incubated for 14 days in a Series 2000 Scientific incubator at 25°C±2. The 6.5 ml volume of the respective extract concentrations and distilled water used in this study was based on the results of the preliminary germination trials. Similarly, the five aqueous extract concentrations were prepared according to the method adopted by Ashrafi et al. (2008).

Data collection

The Petri dishes were left in the incubator for the first 72 h following which germination was recorded daily over a period of 11 days. A seed is considered germinated when its protruded radicle length is about 5 mm within the 14 day specified period. Growth variables such as radicle and plumule elongations were evaluated by separately measuring the radicle and plumule lengths of 15 samples randomly selected from each replicate. After the 14-day duration of the experiment, seed germination inhibition (%) was calculated using the method described by Jefferson and Pennacchio (2003). Decreases in the radicle and plumule lengths of all the extract treated seedlings relative to the control were all calculated.

4.4 Experiment 4: Allelopathic effect of the dried shoot residue of *A. arctotoides* on the growth, morphology and chlorophyll accumulation in tomato and cabbage leaves

The experiment was conducted between January and June 2010 in the greenhouse of the University of Fort Hare, Alice Campus (30°00´-34°15´S and 22°45´-30°15´E). The

greenhouse micro environment including day/night temperatures and relative humidity were automated and their mean values were calculated on daily bases. The photoperiod was most often 16 / 8 h day and night during the experiment.

Seedling establishment

The seedlings of tomato (*Lyncopersicum esculentum* cv. Heinz) and Cabbage (*Brassica oleraceae* cv. Drumhead) were selected for the present study due to their responses to shoot and root aqueous extract treatments and short growth habits. The seeds of the two vegetables were germinated separately in nursery trays, each having 120 holes filled with hygromix soilless media. Culturally, two seeds were planted per hole at 15-25 mm dept and all the trays were irrigated to field capacity using a hand held nozzle sprayer fitted into a water tap. Two weeks after emergence, the seedlings were thinned to one plant per hole followed by the application of a mixture of hydroponic (foliar) nutrient solutions (Aqua-fert 1 and 2) every five days at the rate of 25 ml/ 5L of water. The chemical compositions of the two nutrient solutions were presented in Table 4.4.1.

Table 4.4.1: Chemical compositions of hydroponic nutrient solutions (%)

Mineral elements	Aqua-fert 1 (%)	Aqua-fert 2 (%)
N	7.5	1.5
P	-	2.5
K	8.58	3.4
Ca	6.0	-
Mg	1.5	-
S	-	0.17
Fe	-	0.20
Mo	-	0.08
B	-	0.075
Cu	-	0.005
Zn	-	0.003
Mo	-	0.005

Source: Hygrotech chemicals-Pretoria, South Africa

Key: Aqua-fert = aqua fertilizer

Soil and goat manure characteristics

Sandy loam soil sample from 0-15 cm layer and goat manure collected from Research and Dairy Farms of University of Fort Hare were gathered in heaps, mixed thoroughly and air-dried for two weeks on a raised platform in the greenhouse. Air-dried samples were sieved through 20 mm screen and homogenized. Three kg of sandy loam soil was weighed separately and added into 30 cm polythene pot. Using the method adopted by Awodun et al. (2007), 15 g of goat manure was mixed thoroughly with the soil to boost its fertility. A small sub-sample of the soil and goat manure was composited for physico-chemical property, particle size, textural class and pH. The pH of the mixture was measured in water extract using a glass electrode meter (Model pH 330 SET-1, 82362). Other elemental analyses such as total N, P and K were carried out using wet digestion (Okalebo et al., 2002). The physico-chemical properties of the soil and goat manure were presented in Table 4.4.2.

Greenhouse experiment and addition of the dried shoot residue into the potted soils

Application of *A. arctotoides* ground shoot residue into the potted soils was initiated when the tomato and cabbage seedlings were matured for transplanting (4-week-old). The study was a two factor experiment consisting of two vegetables and three levels of residue concentrations plus a non treated control. The control together with the three residue concentrations namely, 10, 20, and 40 g incorporated into 3 kg soil per pot were hereafter referred to as groups A, B, C and D respectively. The four treatment groups were replicated five times and laid out in a completely randomized design in the

greenhouse. The two vegetable transplants were monitored closely and watered adequately as necessary.

Table 4.4.2: Soil particle size (%) and selected physico-chemical properties of soil and goat manure mixture (%) used in this study

Particle size	(%)
Sand (0.02-2 mm)	68.70
Silt (0.002-0.02 mm)	13.85
Clay (< 0.002 mm)	17.45
Textural class	Sandy loam
Soil and goat manure mixture	
Organic matter (%)	69.6
Total N (%)	5.34
Total P (g kg^{-1})	4.11
Total K (g kg^{-1})	7.23
pH water (1:5)	6.8

Data collection

Prior to transplanting, data were collected from the five randomly selected vegetable seedlings for baseline information and subsequently, at 1, 2, 3 and 4 weeks after transplanting. Growth parameters such as plant height, ground line stem girth, number of leaves, leaf length and width were measured. Leaf area (A) per individual plant was calculated using the method of Pearcy et al. (1989). Immediately after plant harvest, the fresh shoot weight was recorded and the dry weight was obtained at 65°C for 72 h. Data on root growth variables including the length, surface area and dry weight were collected at 4 weeks after transplanting.

The root samples, after harvesting, were washed over a 53µm sieve and thereafter stained with 0.001% methyl violet solution for 24 h to facilitate clear image analysis. Samples were preserved in 30% ethanol and stored at 4^{o}C in the cold room until ready for measurement. The stained roots were suspended in a thin layer of water on a glass tray attached to an HP flatbed Scanjet G3110 scanner to obtain Joint Photographic Expert Group (JPEG) images. Both the root length and surface area measurement were captured using a Medealab Count and Classify Image Analyses Software (MTG Vertrieb GmbH, Altdorf, Germany). The dry root weight was obtained at 65 ^{o}C after 48 h. Chlorophyll pigments and carotenoid contents in the leaves of the two vegetable plants were determined by homogenizing one gram of the freshly harvested green tissue in 10 ml of 80% acetone at 4°C and centrifuged at 2500xg for 10 min. The absorbance of chlorophyll *a*, *b* and carotenoid contents were measured using spectrophotometer at 662, 646, and 470 nm respectively as described by Lichtenthaler and Wellburn (1983).

4.5 Experiment 5: Allelopathic effects of the dried shoot residue of *A. arctotoides* on yield and yield components of tomato and cabbage transplants

Data collection

At 12 weeks after transplanting, tomato yield was evaluated based on the number of fruits, average fruit weight as well as fresh and dry fruit weight per individual treatment group. The economic yield of cabbage was evaluated based on the fresh and dry head weight and total number of leaves per plant at 12 weeks after transplanting. The dry stem and leaf weight of the two vegetables was also considered as the yield component during the period.

4.6 Experiment 6: Allelopathic effects of the dried shoot residue of *A. arctotoides* on nutrient uptake by the two vegetable leaves and soil residual mineral content

Data collection

Data on mineral nutrient uptake were collected at 4 and 12 weeks after transplanting by harvesting the youngest matured leaves from the 4^{th} and 8^{th} branches plus 4^{th} and 6^{th} clusters of tomato and cabbage respectively (Jones et al., 1971). Samples were oven-dried to constant weight at 65 °C and ground to smooth powdery material using a hammer mill machine with a 1 mm mesh sieve. Three replicates of 0.3 g of the ground material were digested separately using the procedures for the determination of mineral elements adopted by Okalebo et al. (2002). Analysis of both macro and micro elements such as K, Ca, Mg, Na, Cu, Fe and Zn was carried out using Inductive Couple Plasma – Optical Emission Spectrometry (1CP-OES) detection, Varian 710-ES series. The absorbance of each mineral element was recorded at the corresponding wavelengths 766.5, 396.8,

285.2, 589, 327.4, 238.2 and 213.8 respectively. Phosphorus was determined on a calorimetric molybdenum / ascorbic acid blue colour development procedure (Okalebo et al., 2002). Total nitrogen was obtained using LECO TruSpec C/N auto analyzer (LECO Corporation, 2003). Analysis of soil residual nutrient elements was determined by air-drying the soil samples collected after the fruit harvest for 2 weeks, ground and pulverized. Three replicates were collected per treatment and kept in well labeled zip lock polythene bags until needed. The soil residual mineral nutrients were determined using the procedures described for leaf mineral analysis Okalebo et al. (2002).

4.7 Statistical Analysis

Data on seed germination and seedling growth as well as plant growth parameters evaluated from the shoot residue treatments were both subjected to one way analysis of variance (ANOVA) using four replicates per treatment. Three replicates of the leaf samples were equally analyzed for chlorophyll pigment accumulation using ANOVA. Treatment means were separated by Duncan Multiple Range Test at 5% probability level using Statistical Analysis System (SAS, 1999) program.

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CHAPTER FIVE

RESULTS

CHAPTER FIVE

5.0 RESULTS

5.1 The foliar morphology of *A. arctotoides* using the Scanning Electron Microscope

The micrographs of the Scanning Electron Microscope (SEM) revealed that anisocytic stomata, glandular trichomes (GTs) and non-glandular trichomes (NGTs) were the three major ultra structures present on both lower (abaxial) and upper (adaxial) surfaces of the leaf of *A. arctotoides* (Figures 5.1A, 5.1B and 5.1C). The anisocytic stomata were located on the epidermal cells below the two trichomes, both the stomata and GTs were more on the abaxial than the adaxial surfaces of the leaf (Figures 5.1A i and ii). The GTs were in addition densely distributed over the abaxial surfaces of the leaf and joined together by two or more fiber-ends of NGTs. Morphologically, the GTs were peltate and uniseriate with globular heads. The globular heads were found within the invagination of the adjacent epidermal cells (Figure 5.1A iv).

On the contrary, the NGTs were filamentous, highly clustered and linearly arranged along the midrib of the leaves (Figure 5.1B i and ii). The filament had a long stalk with variable number of cells attached to the basal segment (Figure 5.1B iii). Besides the three main ultra structures, some crystal deposits were also observed around the stomata and globular head of the GTs (Figure 5.1C i and ii). The energy dispersive X-ray spectroscopy of the spectra showed that Na, Mg and Ca were the major elemental constituents of the crystals (Figure 5.1C iii).

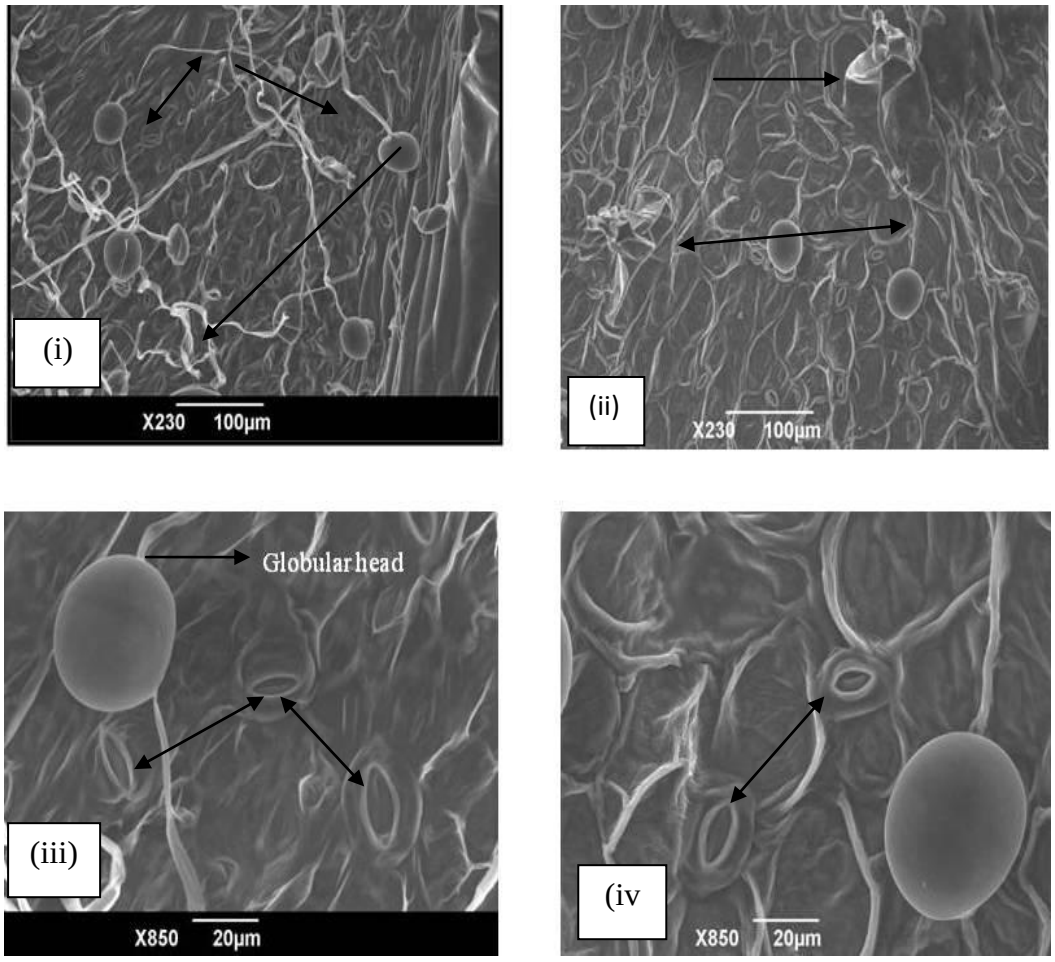


Figure 5.1A: The arrows show (i) the distance between the stomata, (ii) distribution and density on the abaxial and adaxial surfaces of *A. arctotoides.* Magnified view of abaxial (iii) and adaxial (iv) surfaces of the leaf showing the globular heads of GTs.

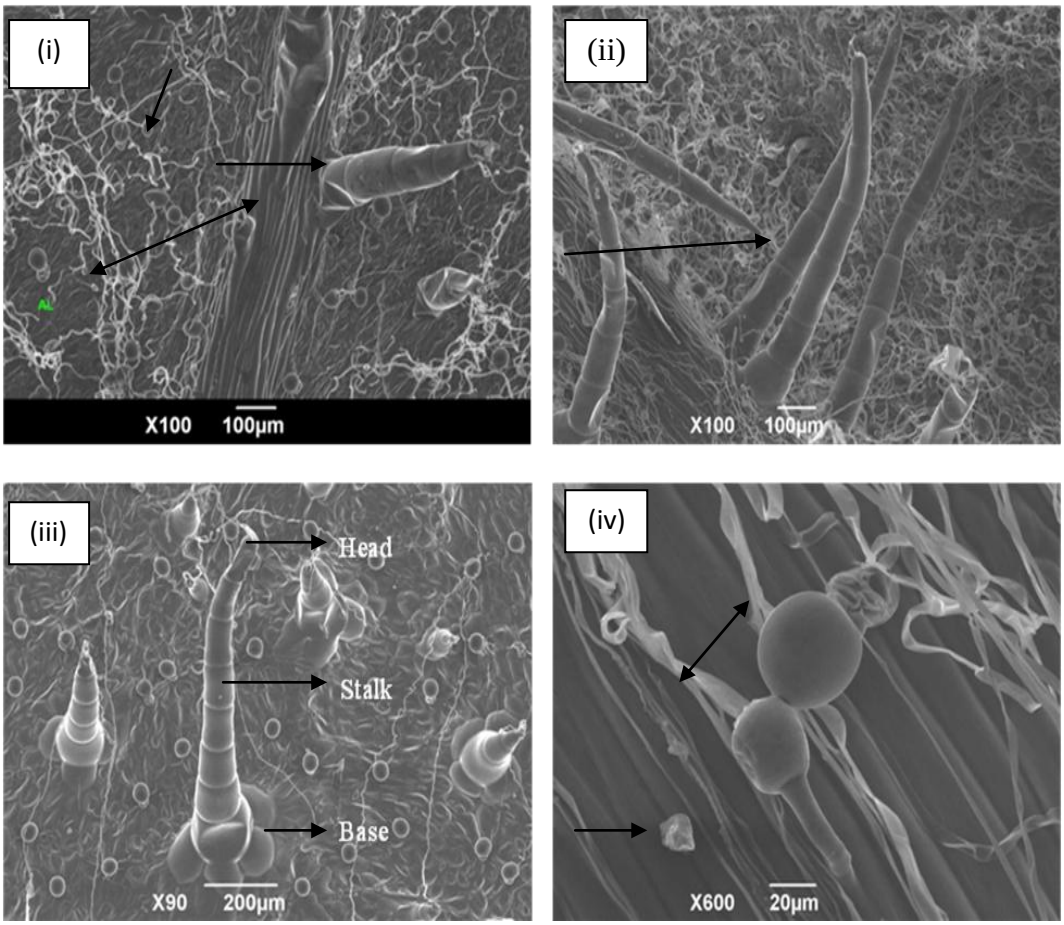


Figure 5.1B: The arrows show distribution and arrangement of glandular (GT) and non glandular (NGT) on the abaxial (i) and (ii) adaxial surfaces of the leaf of *A. arctotoides*. Morphology of NGT showing the head, stalk and base (iii). Note the connections of the GTs by the fibre-ends of NGT (arrowed) on the abaxial surface of the midrib (iv).

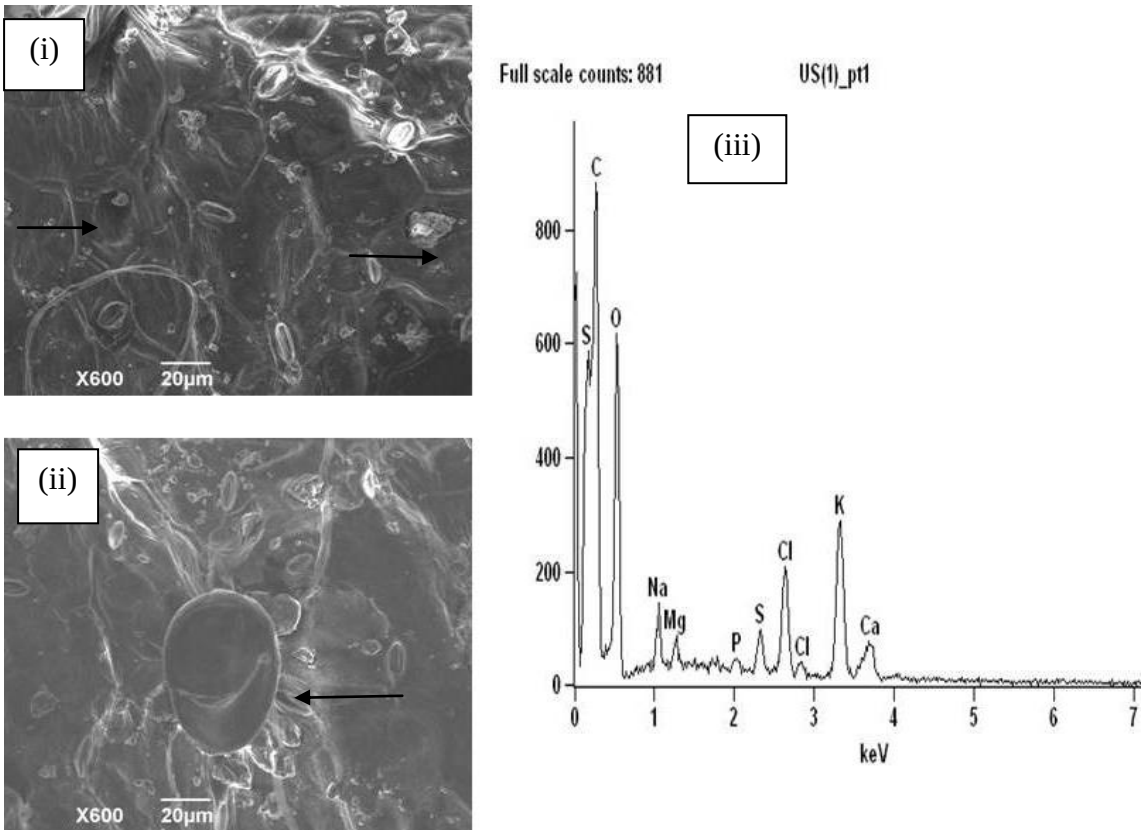


Figure 5.1C: The arrows show the crystal deposits around the stomata (i) and near the globular head of GT (ii) all on the leaf surface of *A. arctotoides*. Energy dispersive X-ray spectroscopy of the crystals (iii).

5.2 Phytochemical screening and percentage composition of the crude chemical constituents of the root and shoot aqueous extracts of *A. arctotoides*

The results of the broad chemical compositions and percentage crude yields of selected phytochemical constituents of the root and shoot samples of *A. arctotoides* are presented in Table 5.2. Qualitative analysis of the two plant parts revealed the presence of secondary metabolites such as alkaloids, flavonoids, phenolics, saponnins, tannins and triterpenes. Additionally, cardiac glycosides and steroids were also detected in the shoot

but they were absent in the root. On the other hand, quantitative estimation of the crude yields of selected chemical constituents of the two plant parts of *A. arctotoides* demonstrated that the root sample had higher percentage of alkaloid (0.97%) than the shoot (0.64%). Conversely, the amount of flavonoids detected in the shoot of the plant was about triple folds (1.02%) of that observed in the root (0.35%). Other secondary metabolites including; phenols and tannins evaluated in this study were relatively fairly distributed in the two plant parts. However, the percentage compositions of the two compounds were found to be higher in the shoot than the root.

Table 5.2: Phytochemical screening and percentage composition of selected crude yields of the root and shoot samples of *A. arctotoides*

Phytochemical constituents	Root	Shoot	Root (%)	Shoot (%)
Alkaloid	+	+	0.97±0.04	0.64±0.02
Cardiac glycosides	-	+	-	-
Flavonoids	+	+	0.35±0.03	1.02±0.07
Phenols	+	+	0.18±0.02	0.26±0.08
Saponins	+	+	-	-
Steroids	-	+	-	-
Tannins	+	+	0.08±0.01	0.12±0.02
Triterpenes	+	+	-	-

Key: (+) = presence (-) = absence

5.3 Allelopathic effects of the root and shoot aqueous extracts of *A. arctotoides* on seed germination, radicle and plumule growth of cabbage, carrot, tomato and spinach

Preliminary optimum water requirement and seed viability tests

The results of the tetrazolium seed assessment revealed that the viability percentages of cabbage, carrot, spinach and tomato seeds used in this study were 96, 92, 71 and 100% respectively. This implied that all the seeds were viable. Similarly, the results of the preliminary germination experiments for optimum water requirement showed that on the average, the highest number of germinated seeds from the four vegetables were from the Petri dishes moistened with 6.5 ml of distilled water. Hence, the choice of the volume (6.5 ml) in the various extracts concentrations for the moistening of the seeds throughout the experiments.

Effects of the root and shoot aqueous extracts of A. arctotoides on the germination of vegetable seeds

The addition of the root and shoot aqueous extracts of *A. arctotoides* significantly ($P < 0.05$) reduced the germination of the four vegetable seeds after 14 days of incubation. The root extract at 10 mg/ml inhibited the germination of cabbage, tomato, carrot, and spinach seeds by 84.0, 83.2, 72.8 and 37.4% respectively (Figure 5.3.1A). However, spinach seeds incubated with the extract at 8 mg/ml also had equal percentage reduction with 10 mg/ml. The effects of the root extract at 2, 4 and 6 mg/ml varied significantly among the four seeds even though the germination inhibition on tomato and carrot seeds treated with 2 and 4 mg/ml of the extract were not significantly different. Unlike the root,

application of the shoot extract at 10 mg/ml resulted in complete failure of the seeds of cabbage and carrot to germinate. However, tomato and spinach seeds were suppressed by 91.5 and 61.2% respectively (Figure 5.3.2B). The same extract at 6 and 8 mg / ml also had higher percentage reductions on the germination of the four vegetable seeds but there were no significant differences in the two spinach seeds treated with the shoot extracts at 2 and 4 mg / ml.

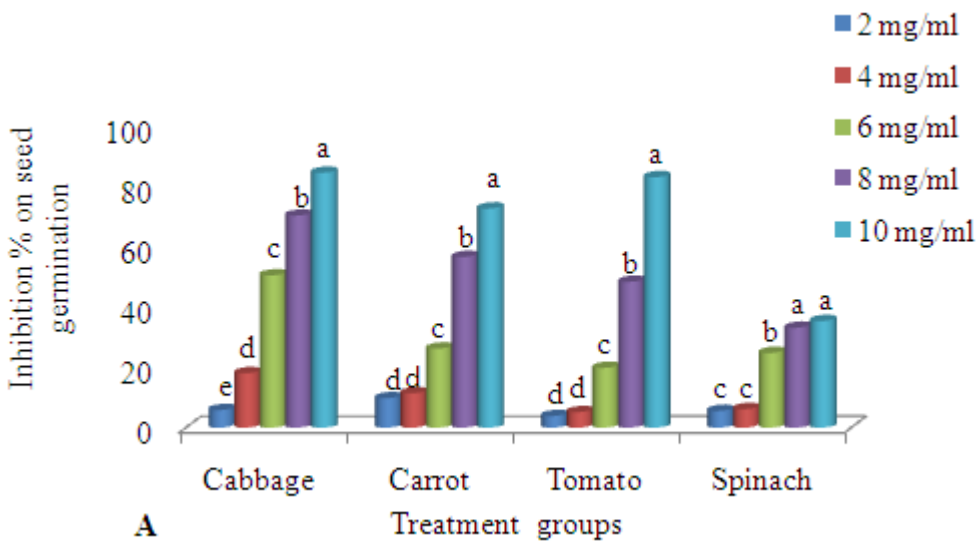
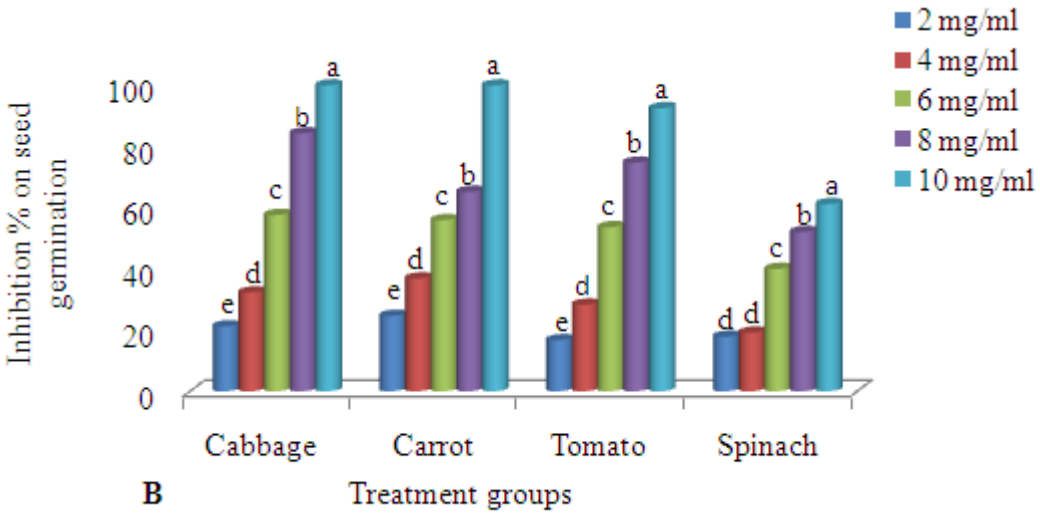


Figure 5.3.1A: Percentage inhibition on the germination of vegetable seeds as influenced by the aqueous extract of the root of *A. arctotoides*



Figures 5.3.1B: Percentage inhibition on the germination of vegetable seeds as influenced by the aqueous extracts of the shoot of *A. arctotoides*

Effects of the root and shoot aqueous extracts of A. arctotoides on the radicle length of the vegetable seedlings

The effects of the root and shoot aqueous extract of *A. arctotoides* on radicle growth of cabbage, carrot, tomato and spinach incubated with the two plant extracts at all concentrations followed similar trend with seed germination inhibition. The reductions in radicle length of the vegetable seedlings caused by addition of the root extract at 10 mg/ml ranged between 92.6 to 83.4% (Figures 5.3.2A). The extract at 8 mg/ml also resulted in more than 50% inhibition on the studied growth parameter of cabbage, carrot, tomato and spinach incubated with the treatment. However, the root extract at 4 and 6 mg/ ml plus 6 and 8 mg/ml on the radicle length of tomato and spinach respectively were not significantly different from each other. On the contrary, application of the shoot extract at 10 mg/ml reduced the radicle length of tomato and spinach by 94.1 and 89.7% respectively (Figure 5.3.2B).

Apart from the declines in the length, the tips of cabbage and tomato radicle incubated in the two extracts at 8 and 10 mg/ml appeared brownish in colour and thicker in diameter than those treated with lower extract concentrations.

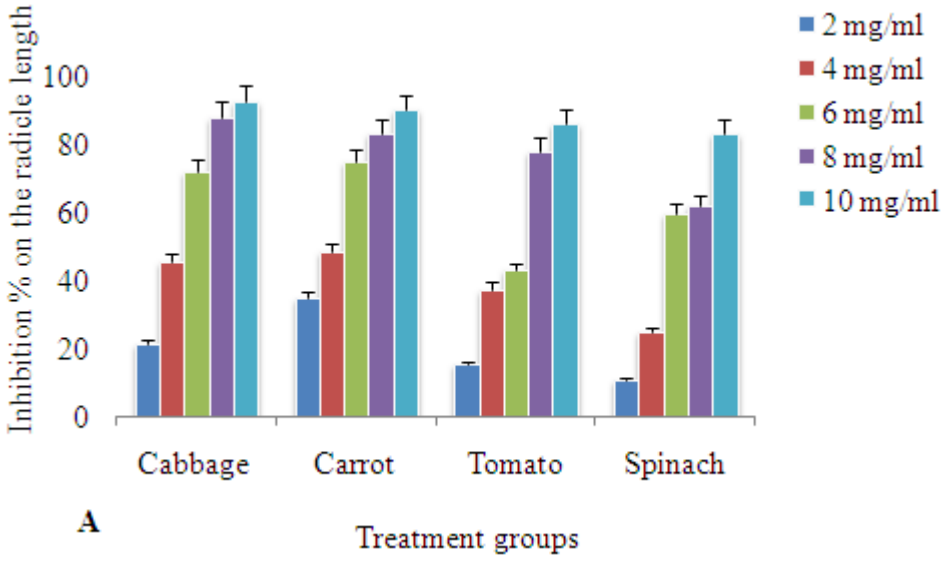


Figure 5.3.2A: Percentage inhibition on the radicle length of the four vegetables as influenced by the aqueous extracts of the root *of A. arctotoides*

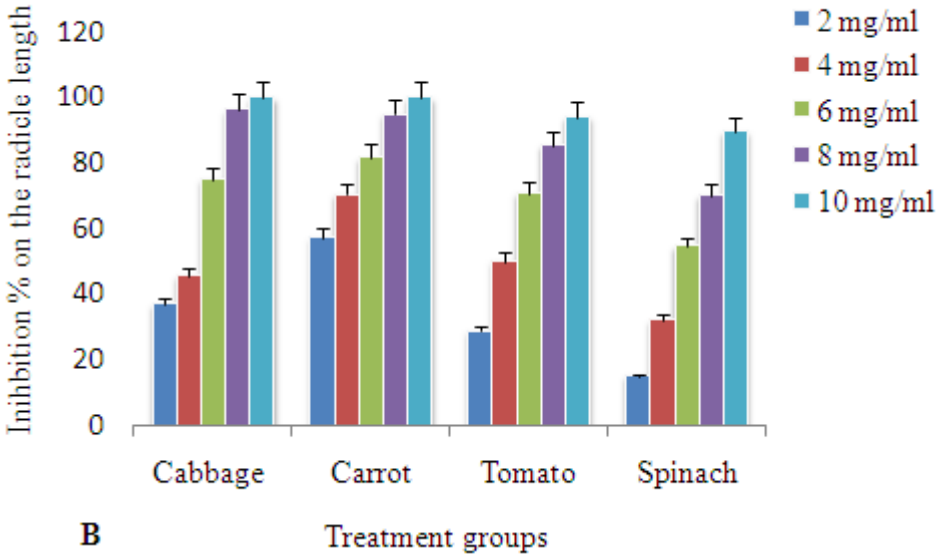


Figure 5.3.2B: Percentage inhibition on the radicle length of the four vegetables as influenced by the aqueous extracts of the shoot *of A. arctotoides*

Effect of the root and shoot aqueous extracts of A. arctotoides on plumule growth of vegetable seedlings

The reductions in the plumule growth of cabbage, carrot, tomato and spinach treated with the root and shoot aqueous extracts of *A. arctotoides* followed the same trend with those of seed germination and radicle length. However, when compared with the radicle length, the plumule growth generally appeared to be less affected by the treatments (Figure 5.3.3A). The root extract at 8 and 10 mg/ml considerably reduced the plumule length of all the vegetables whereas the two lowest concentrations had equal inhibition percentages on the plumule growth of all the vegetables except for carrot. The shoot extract on the other hand caused highly significant differences in the plumule growth of all the treated crops at the varying concentrations (Figure 5.3.3B). While the extract at 10 mg/ml had 100% inhibition on the plumule length of tomato that of spinach at the same extract concentration was reduced by 82.3%. Generally, the effects of the shoot extract at 2, 4, 6 and 8 mg / ml on the plumule growth of the four vegetables were significantly higher than those recorded for root extract at similar concentrations.

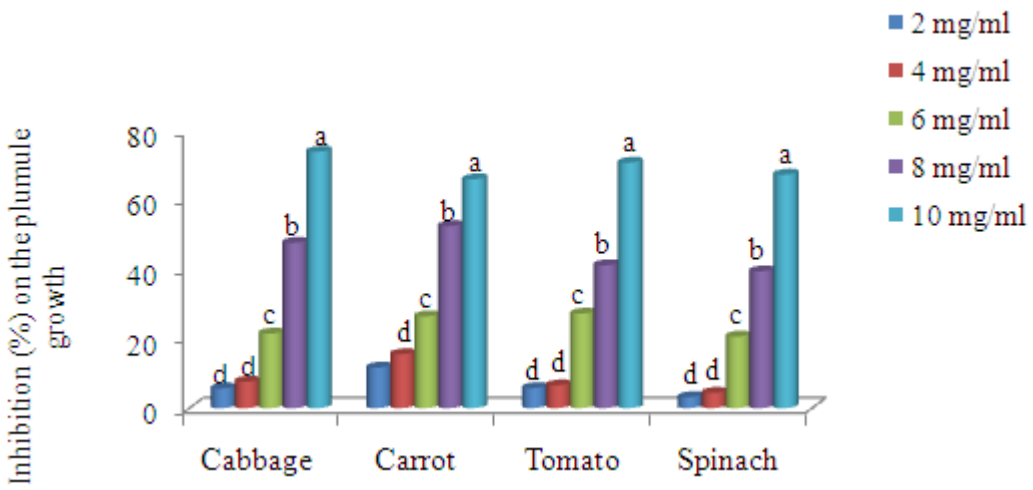


Figure 5.3.3A: Percentage inhibition on plumule growth of the four vegetables as influenced by the aqueous extracts of the root *of A. arctotoides*

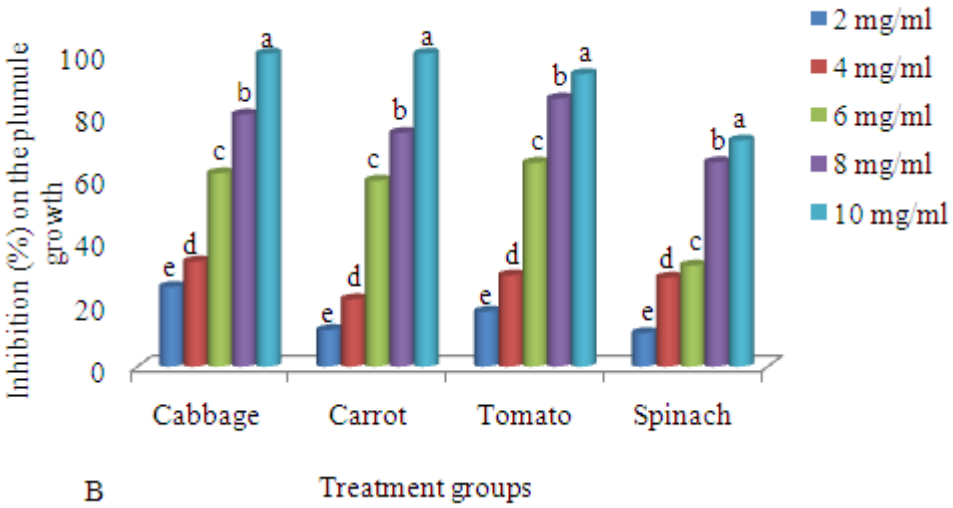


Figure 5.3.3B: Percentage inhibition on the plumule growth of the four vegetables as influenced by the aqueous extracts of the shoot *of A. arctotoides*

5.4 Allelopathic effect of the dried shoot residue of *A. arctotoides* on the growth, morphology and chlorophyll content of tomato and cabbage leaves

Effects of shoot residue on plant height and stem girth

There were no significant differences between the height of tomato plants grown with the shoot residue of *A. arctotoides* and the control throughout the duration of the experiment except at 4 weeks after transplanting (Table 5.4.1A). During the period, the height of all tomato transplants in treatments B, C and D differed considerably in comparison with the control. No significant variation was observed between the height of treatments C and D. Similarly, the height of cabbage plants grown in the amended soils was not significantly ($P < 0.05$) different throughout the experiment except at 3 weeks after transplanting. Relative to control, the height of plants treated with the amended soils decreased with increase in shoot residue concentrations but those of cabbages under treatments B and C was equal (Table 5.4.1A).

Similar to the plant heights, the shoot residue added to the soil had no effects on the stem girth of all tomato groups (B, C and D) at 1 and 2 weeks after transplanting (Table 5.4.1B). However, during the last two sampling periods, significant differences were recorded between the ground line stem diameter of the control and those grown in residue treated soils. In spite of the observed differences at 3 weeks after transplanting, the stem diameter of plants in treatments C and D was equal. Also at 4 weeks after transplanting, similar observation was noticed but with treatment B having significantly higher stem diameter as the control. Those of treatments C and D also had equal but the lowest stem girth values. Among the cabbage plants, there was no variation between the stem girth of

the control and those grown with the shoot residue at 1 and 2 weeks after transplanting (Table 5.4.1B).

The stem diameter of cabbage plants in treatments B and C was not statistically ($P < 0.05$) different from the control at 3 weeks after transplanting. That of treatment D also compared favourably well with treatment C during the period. Moreover, at 4 weeks after transplanting, no significant differences were noticed between the stem girth of cabbage plants in the control and the least residue treated soils. Those of treatments C and D also followed similar trend.

Table 5.4.1A: Allelopathic effects of the dried shoot residue of *A. arctotoides* on the plant height (cm) of tomato and cabbage

	Tomato				Cabbage			
	Sampling periods (week)							
Treatments	1	2	3	4	1	2	3	4
A	18.33±2.13[a]	16.67±1.50[a]	20.71±2.15[a]	32.97±2.84[a]	11.10±1.33[a]	11.00±1.24[a]	10.27±1.17[a]	9.83±0.94[a]
B	18.67±2.23[a]	16.73±1.13[a]	19.50±1.68[a]	27.82±2.84[b]	8.36±1.02[a]	8.73±1.08[a]	8.60±1.10[b]	8.63±1.16[a]
C	16.77±1.74[a]	16.23±1.08[a]	19.42±1.82[a]	20.01±2.19[c]	11.93±1.45[a]	10.27±1.26[a]	8.35±0.94[b]	8.57±1.09[a]
D	19.63±1.85[a]	15.02±1.24[a]	19.70±2.07[a]	19.61±2.23[c]	11.40±1.12[a]	9.47±1.12[a]	6.74±0.82[c]	7.60±0.76[a]

Data are means (± S.D). N=4; Mean values followed by the same letter along a column are not significantly different at P > 0.05

Key: A = Control; B = 10 g; C = 20 g; D = 40 g residue

Table 5.4.1B: Allelopathic effects of the dried shoot residue of *A. arctotoides* on the stem girth (cm) of tomato and cabbage

	Tomato				Cabbage			
	Sampling periods (week)							
Treatments	1	2	3	4	1	2	3	4
A	0.68±0.09[a]	1.09±0.11[a]	2.34±0.14[a]	2.56±0.29[a]	0.95±0.06[a]	1.50±0.10[a]	2.18±0.14[a]	3.56±0.42[a]
B	0.74±0.05[a]	1.05±0.11[a]	2.46±0.14[b]	2.62±0.17[a]	0.97±0.08[a]	0.95±0.08[a]	2.22±0.12[a]	3.63±0.45 [a]
C	0.72±0.08[a]	0.98±0.10[a]	1.83±0.19[c]	2.38±0.24[b]	0.93±0.08[a]	1.04±0.12[a]	2.03±0.10[ab]	2.86±0.30[b]
D	0.70±0.10[a]	1.12±0.06[a]	1.65±0.14[c]	2.14±0.21[b]	0.90±0.07[a]	0.88±0.05[a]	1.42±0.11[b]	2.68±0.18[b]

Data are means (± S.D). N=4; Means with the same letter along the column are not significantly different at P > 0.05

Key: A = Control; B = 10 g; C = 20 g; D = 40 g residue

Effects of shoot residue on leaf morphology and number of leaves

The addition of the shoot residue of *A. arctotoides* into the potted soils significantly ($P < 0.05$) affected the number of tomato and cabbage leaves. At 2 weeks after transplanting, chlorosis and necrotic symptoms were obvious in the two vegetable transplants grown in the amended soils. These morphological changes as expressed in form of yellowish colouration and irregular white patches were more along the leaf margin of tomato and on cabbage leaf blade and leaf apex. At higher residue concentrations (treatments C and D), wilting and drying of matured leaves were also common in the two crops. These symptoms, however, gradually disappeared at 3 and 4 weeks after transplanting with rapid increase in the number of leaves per individual plant.

When compared with the control, the shoot residue significantly ($P < 0.05$) reduced the number of tomato and cabbage leaves during the study. At 2 weeks after transplanting, the number of leaves per tomato transplants in treatments B, C and D were not significantly ($P < 0.05$) different (Table 5.4.2). At 3 weeks after transplanting, the number of leaves per individual tomato treated plant decreased as the concentration of the shoot residue increased. At 4 weeks after transplanting, no significant difference was recorded between the number of leaves found on cabbage seedlings in the control and treatment B. Treatment groups C and D were not significantly ($P < 0.05$) different but with lesser number of leaves. The effects of the shoot residue also had similar effects on the number of leaves per cabbage transplant under the treatments (Table 5.4.2). At 2 and 3 weeks after transplanting, the number of leaves in treatment B was similar to the control (treatment A), those of C and D also had statistically equal number

of leaves but not comparable with the control. Similar trend was observed at 4 weeks after transplanting with treatment D having significantly the least number of leaves among the group.

Table 5.4.2: Allelopathic effects of the dried shoot residue of *A. arctotoides* on the number of tomato and cabbage leaves

	Tomato			Cabbage		
	Sampling periods (week)					
Treatments	2	3	4	2	3	4
A	28.4±2.50[a]	41.6±3.10[a]	63.5±3.27[a]	4.2±0.10[a]	9.4±0.63[a]	14.5±1.13[a]
B	21.2±1.81[b]	37.4±2.80[b]	64.6±2.65[a]	3.4±0.08[ab]	6.5±0.50[b]	12.4±1.02[ab]
C	19.8±2.03[b]	34.8±2.68[c]	57.8±1.79[b]	2.6±0.08[b]	4.5±0.12[c]	11.5±1.08[b]
D	20.8±1.76[b]	26.2±2.11[d]	58.1±3.02[b]	2.4±0.07[b]	4.0±0.10[d]	8.8±0.74[c]

Data are means (± S.D). N=4; Means with the same letter along the column are not significantly different at P > 0.05

Key: A = Control; B = 10 g; C = 20 g; D = 40 g residue

Effects of shoot residue on leaf area

One week after transplanting, there was no observable variation in the leaf area of both treated and controlled tomato transplants. The leaf area of those vegetables under treatments B, C and D decreased with increases in shoot residue concentrations added to the soil at 2, 3 and 4 weeks after transplanting (Figure 5.4.1A). At 4 weeks after transplanting, the leaf area of treatments B and C was not significantly different. When compared with the control, the leaf area of all tomato transplants grown with treatment groups B, C and D were inhibited by 25.9, 42.3and 51.2% respectively. Unlike tomato transplants, the leaf area of all cabbages (both the control and treated) were not significantly different during the first two weeks after transplanting (Figure 5.4.1B). However, significant variations were noticed among all transplants grown in the treated soils. The inhibition percentages in the leaf area of cabbage as influenced by the addition of dried shoot residue at the three concentrations were 31.6, 64.3 and 74.4 respectively.

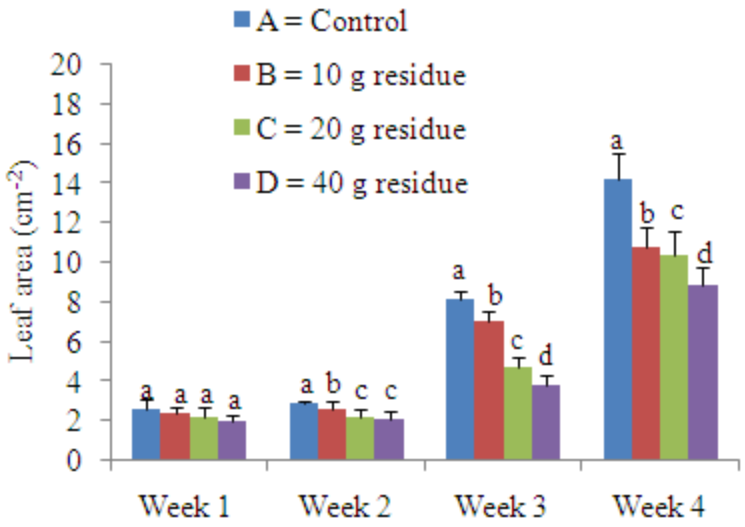


Figure 5.4.1A: Effects of the dried shoot residue of *A. arctotoides* on the leaf area (cm^{-2}) of tomato

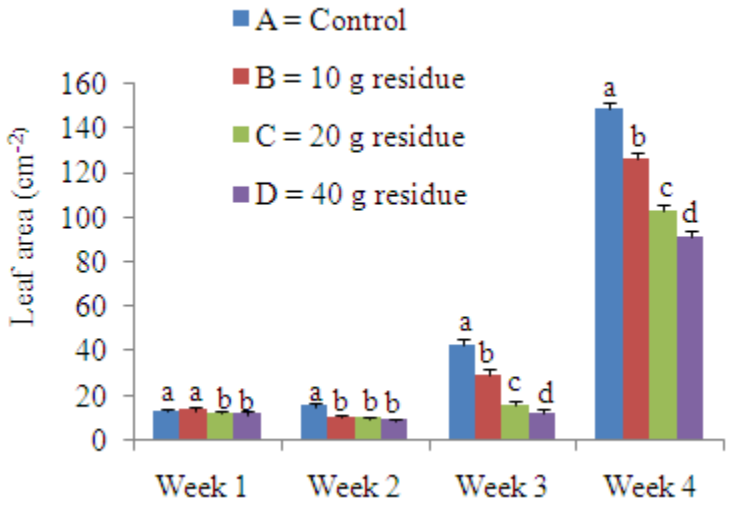


Figure 5.4.1B: Effects of the dried shoot residue of *A. arctotoides* on the leaf area (cm^{-2}) of cabbage

Effects of shoot residue on the fresh and dry shoot weight

Application of the shoot residue of *A. arctotoides* significantly suppressed the fresh and dry shoot weights of tomato and cabbage transplants. The fresh shoot weight of tomato in treatments B and C were not significantly different from the control at 2 weeks after transplanting (Table 5.4.3A). Similarly, the fresh shoot weight of the transplants in treatment D was not significantly different from those of groups B and C. At 3 and 4 weeks after transplanting, the fresh shoot weight of tomato transplants grown in the residue amended soils decreased as treatment concentration increased. However, at 3 weeks after transplanting, there were no significant differences in treatments C and D. The dry shoot weights of treatments B, C and the control followed similar trend at 2 weeks after transplanting (Table 5.4.3A). At 3 and 4 weeks after transplanting, the dry shoot weights of treatments C and D together with B and C were not significantly different. The reductions caused by the three shoot residue concentrations in the dry shoot weight of tomato at 4 weeks after transplanting were 38.6, 45.5 and 70.3% respectively.

On the contrary, the fresh shoot weight of all cabbage transplants treated with the shoot residue differed significantly in comparison with the control throughout the sampling duration except at 2 weeks after transplanting (Table 5.4.3B). The fresh shoot weight of the transplants in treatment B was significantly lower than treatment C during the period, however, the fresh shoot weight of the control was the highest. The dry shoot weight of the vegetables also followed a similar trend with that of the fresh weight. At 2 weeks after transplanting however, the dry shoot weight of transplants in treatments B and C were similar. The reductions in the dry shoot weight of

cabbage at 4 weeks after transplanting as influenced by shoot residue treatments B, C and D effects were 57.5, 73.3 and 87.5% respectively.

Table 5.4.3A: Allelopathic effects of the dried shoot residue of *A. arctotoides* on fresh and dry shoot weight (g) of tomato

	Fresh shoot weight (g)			Dry shoot weight (g)		
	Sampling periods (weeks)					
Treatments	2	3	4	2	3	4
A	2.08±0.42[a]	5.58.±0.40[a]	15.60±1.90[a]	0.19±0.08[a]	0.63±0.07[a]	1.45±0.11[a]
B	1.84±0.34[ab]	4.26±0.22[b]	11.18±1.44[b]	0.18±0.04[a]	0.45±0.05[b]	0.89±0.10[b]
C	1.78±0.30[ab]	3.21±0.14[c]	8.74±1.25[c]	0.19±0.05[a]	0.38±0.06[c]	0.79±0.08[b]
D	1.62±0.20[b]	3.39±0.17[bc]	6.17±1.12[d]	0.15±0.04[b]	0.36±0.02[c]	0.43±0.03[c]

Data are means (± S.D). N=4; Means followed by the same letter are not significantly different at P > 0.05

Key: A = Control; B = 10 g; C = 20 g; D = 40 g residue

Table 5.4.3B: Allelopathic effects of the dried shoot residue of *A. arctotoides* on fresh and dry shoot weight (g) of cabbage

	Fresh shoot weight (g)			Dry shoot weight (g)		
	Sampling periods (weeks)					
Treatments	2	3	4	2	3	4
A	2.97±0.10[a]	6.23±0.18[a]	11.55±1.46[a]	0.31±0.04[a]	0.50±0.06[a]	1.20±0.10[a]
B	1.61±0.08[c]	2.71±0.15[b]	4.46±0.68[b]	0.20±0.01[b]	0.28±0.05[b]	0.51±0.08[b]
C	2.04±0.10[b]	1.38±0.12[c]	2.40±0.30[c]	0.23±0.02[b]	0.21±0.05[c]	0.32±0.04[c]
D	0.75±0.06[d]	0.53±0.04[d]	1.06±0.12[d]	0.16±0.01[c]	0.12±0.02[d]	0.15±0.04[d]

Data are means (± S.D). N=4; Means followed by the same letter are not significantly different at P > 0.05

Key: A = Control; B = 10 g; C = 20 g; D = 40 g residue

Effects of shoot residue on root morphology, fresh and dry root weight

The root morphology including the root length and root area of tomato and cabbage transplants were adversely affected by the residue treatments at 4 weeks after transplanting. The data for week 4 were selected for the interpretation of results because there were no significant differences among treatments B, C and D on root morphology and dry root weights at 1, 2 and 3 after transplanting. When compared with the control, the addition of the shoot residue at the three concentrations reduced the root length of tomato transplants by 33.6, 68.5 and 81.5% s (Figure 5.4.2A). Cabbage transplants were inhibited by 24.2, 40.9 and 65.7% (Figure 5.4.2B). The data on the dry root weight of the two vegetables also showed highly significant differences between the control and soil amended groups B, C and D. The losses in the dry root weight due to treatment effects at 4 weeks after transplanting were 61.3, 82.9 and 83.4% for tomato and 53.1, 54.7 and 67.2% for cabbage transplants respectively.

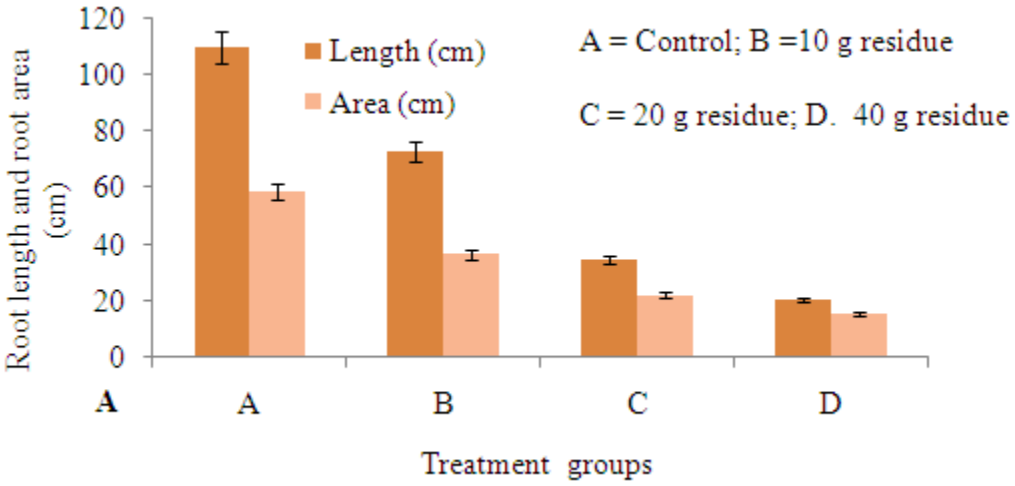


Figure 5.4.2A: Effects of the dried shoot residue of *A. arctotoides* on the root length and root area of tomato

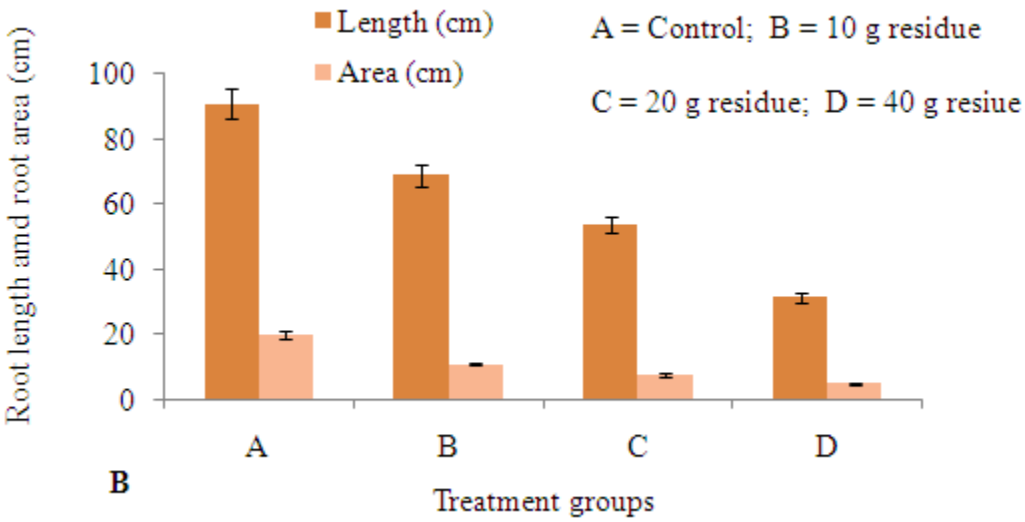


Figure 5.4.2B: Effects of the dried shoot residue of *A. arctotoides* on the root length and root area of cabbage

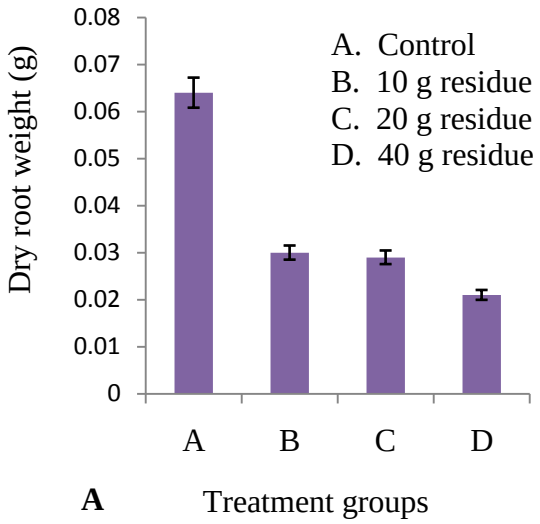


Figure 5.4.3A: Effects of the dried shoot residue of *A. arctotoides* on the dry root weight of tomato

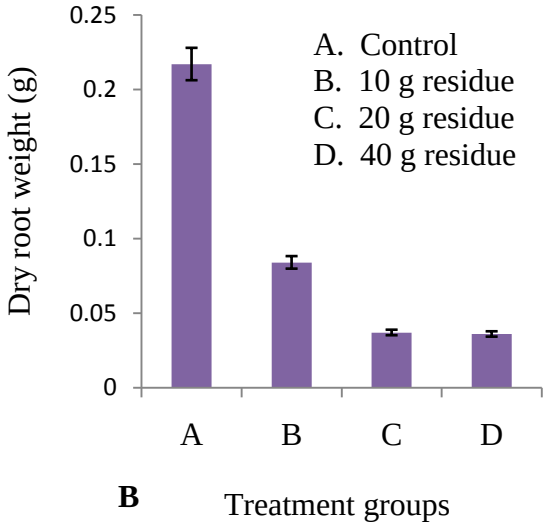


Figure 5.4.3B: Effects of the dried shoot residue of *A. arctotoides* on the dry root weight of cabbage

Effects of shoot residue on chlorophyll a, b and total chlorophyll contents in tomato and cabbage leaves

The result of the chlorophyll pigments accumulation indicated that during the first two weeks of the experiment, the amount of chlorophyll *a* detected in the leaves of tomato transplants grown in the amended soils decreased as the shoot residue concentration increased (Table 5.4.4).). However, there was no significant difference in the mean concentration of the pigment in treatments B, C and D at 2 weeks after transplanting. At 3 and 4 weeks after transplanting, the amount of chlorophyll *a* pigment in the control and treatments B and C were not significantly different. Among the cabbage transplants, the shoot residue of *A. arctotoides* had similar effects on chlorophyll *a* pigment content in the leaves of the vegetable grown with the treatments. The amount of the pigment extracted from the transplants treated with the least residue concentration (treatment B) was significantly higher and equal to the control at 1 and 2 weeks after transplanting (Table 5.4.4). Although, the addition of the shoot residue consistently reduced the quantity of chlorophyll *a* in treatments B, C and D at 3 and 4 weeks after transplanting no significant variation was noticed in the amount of the pigment in treatments B and C at 3 weeks after transplanting.

Except at one week after transplanting, the mean concentration of chlorophyll *b* in the leaves of tomato transplants under the residue treated groups varied significantly throughout the duration of the experiment (Figure 5.4.6A). While no difference was observed in the mean concentration of the chlorophyll recorded for treatments C and D at 2 weeks after transplanting, the amount of

the pigment detected between the control and treatment B followed similar trend but significantly higher than the other treatments at 2 and 3 weeks after transplanting.

Table 5.4.4: Allelopathic effects of the dried shoot residue of *A. arctotoides* on chlorophyll *a* content (mg g^{-1}) of tomato and cabbage leaves

Treatments	Tomato				Cabbage			
	Sampling periods (week)							
	1	2	3	4	1	2	3	4
A	1.58±0.03^{a}	1.73±0.04^{a}	1.80±0.10^{a}	1.81± 0.11^{a}	1.16± 0.05^{a}	1.66±0.12^{a}	1.72± 0.10^{a}	1.65± 0.11^{a}
B	0.63±0.02^{c}	1.49±0.02^{b}	1.75±0.06^{a}	1.81± 0.10^{a}	1.13±0.04^{a}	1.61±0.08^{a}	1.49± 0.05^{b}	1.53± 0.10^{b}
C	0.89±0.01^{b}	1.29±0.01^{b}	1.75± 0.04^{a}	1.81± 0.10^{a}	1.09± 0.02^{b}	1.42± 0.04^{b}	1.46± 0.03^{b}	1.31± 0.06^{c}
D	0.42±0.01^{d}	1.25±0.01^{b}	1.49± 0.01^{b}	1.24± 0.05^{b}	0.86±0.04^{c}	1.17± 0.01^{c}	1.33± 0.05^{c}	1.24± 0.02^{d}

Data are means (± S.D). N=3; Means followed by the same letter along the column are not significantly different at P > 0.

Key: A = Control; B = 10 g; C = 20 g; D = 40 g residue

The quantity of the chlorophyll detected in treatment C was not significantly different from the control at 4 weeks after transplanting (Figure 5.4.6A). Unlike tomato transplants, the mean concentration of chlorophyll *b* pigment in the leaves of all cabbage transplants with the exception of treatment D was similar at one week after transplanting (Figure 5.4.6B). Relative to the control, the quantity of the pigment was maximum in transplants with the least residue concentration (treatment B) at 2 and 3 weeks after transplanting. When compared with the control at 4 weeks after transplanting, the addition of the shoot residue consistently reduced the amount of chlorophyll *b* in treatments B, C and D. However, no significant difference was recorded in mean concentration of the pigment between treatments B and C.

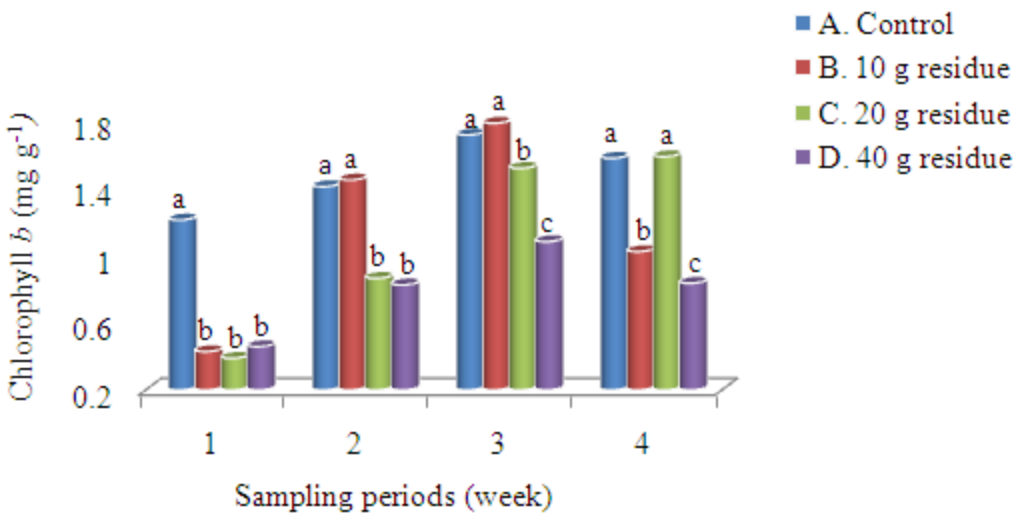


Figure 5.4.4A: Effects of the dried shoot residue of *A. arctotoides* on chlorophyll *b* content in tomato leaves

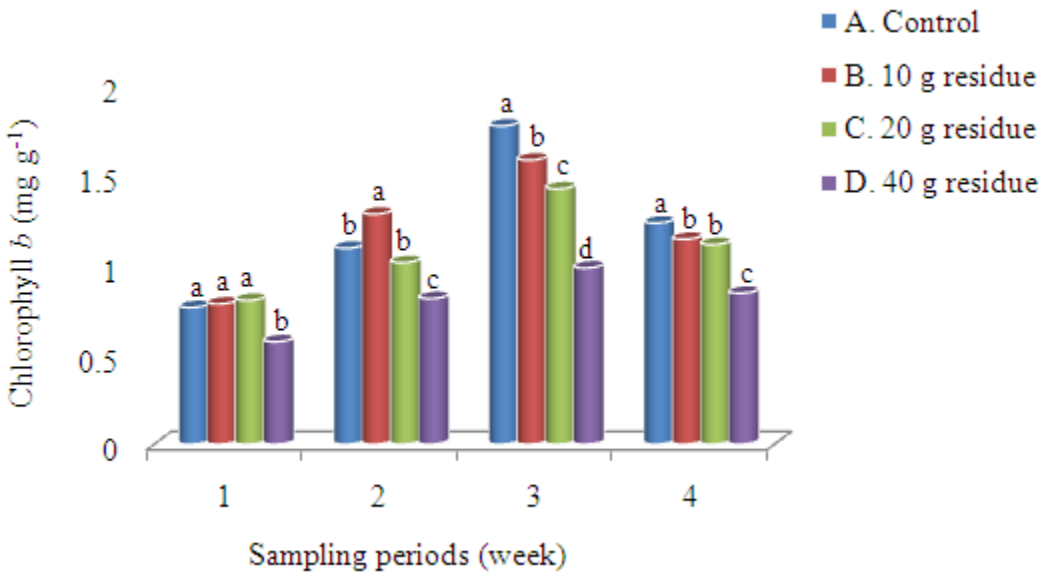


Figure 5.4.4B: Effects of the dried shoot residue of *A. arctotoides* on chlorophyll *b* content in cabbage leaves

5.5 Allelopathic effects of the dried shoot residue of *A. arctotoides* on yield and yield components of tomato and cabbage at 12 weeks after transplanting

Effects of the shoot residue on yield and yield components of tomato and cabbage

The yield and yield components of the two vegetables at 12 weeks after transplanting were significantly (P >0.05) suppressed by the applied *A. arctotoides* shoot residue treatments. The number of fruits, average fruit weight and the total fruit yield of tomato per treatments decreased with increase residue concentration (Table 5.5.1). However, no significant difference was recorded between treatments C and D for the number of fruits and average fruit weight. The effects of the shoot residue of *A. arctotoides* on the dry weight yield of tomato also followed similar trend with treatments C and D having significantly lower values than the control (treatment A). The dry stem and leaf weights of treatments B and C were however, not significantly different from one another (Table 5.5.1).

On the other hand, the effects of the treatments on cabbage yield components showed that the fresh head weight of the vegetable decreased as the concentration of the residue increased (Table 5.5.2). Nonetheless, no difference was recorded between the dry stem weight of the control and treatment B. Similar observation was also noticed between treatments C and D for the same yield component. Biomass accumulation as expressed by the dry leaf and head weight of cabbage followed the same trend with dry stem weight. The results indicated that the leaf biomass dry matter of treatments C and D and the head weight of all residue treated cabbage transplants were not significantly different and lower than the control during the period. In spite of the similarities

among the treatment groups, the total dry shoot weight of the individual treatment decreased with increase in the concentrations of the dried shoot residue of *A. arctotoides* added to the soils.

Table 5.5.1A: Allelopathic effects of dried shoot residue of *A. arctotoides* on tomato fruit yield and dry matter (per treatment) at 12 weeks after transplanting

Treatments	Number of fruits	Average fruit weight (g)	Total fruit yield (g) per treatment	Dry matter weight (g)			
				Stem	Leaf	Fruit	Total
A	8.5±1.61[a]	14.28±1.74[a]	120.92±4.67[a]	15.15±1.20[a]	11.98±1.50[a]	9.58±1.03[a]	36.7±2.50[a]
B	6.0±1.40[b]	14.04±1.80[a]	85.44±3.72[b]	12.73±1.31[b]	9.00±0.76[b]	6.02±0.10[b]	27.75±2.71[b]
C	5.5±0.74[bc]	11.03±1.22[b]	62.75±3.04[c]	12.18±1.14[b]	8.05±0.47[bc]	2.27±0.07[c]	22.49±1.86[c]
D	4.0±0.50[c]	10.00±1.15[b]	40.78±2.81[d]	10.25±1.08[c]	7.20±0.60[c]	1.46±0.03[d]	18.91±2.42[d]

Data are means (± S.D). N=4; Means followed by the same letter along the column are not significantly different at $P > 0.05$

Key: A = Control; B = 10 g; C = 20 g; D = 40 g residue

Table 5.5.1B: Allelopathic effects of dried shoot residue of *A. arctotoides* on cabbage head weight and dry matter (per treatment) at 12 weeks after transplanting

Treatments	Fresh head weight (g)	Stem	Leaf	Head	Total
			Dry matter weight (g)		
A	20.29±2.03[a]	1.86±0.04[a]	16.50±1.48[a]	2.17±0.02[a]	20.52±2.22[a]
B	14.40±1.59[b]	1.59±0.01[a]	12.67±1.32[b]	1.50±0.01[b]	15.36±2.03[b]
C	8.67±1.12[c]	1.30±0.02[b]	9.54±0.80[c]	1.04±0.00[b]	11.63±1.49[c]
D	7.06±1.07[d]	1.25±0.01[b]	7.88±0.74[c]	0.60±0.00[b]	9.54±1.12[d]

Data are means (± S.D). N=4; Means followed by the same letter along the column are not significantly different at $P > 0.05$

Key: A = Control; B = 10 g; C = 20 g; D = 40 g residue

5.6 Allelopathic effects of dried shoot residue of *A. arctotoides* on the mineral uptake by tomato and cabbage leaves and soil residual nutrients content

Effects of the dried shoot residue on mineral uptake by tomato leaves at 4 and 12 weeks after transplanting

The mineral content of the leaves of the two vegetable transplants varied significantly among the treatments and between the two sampling periods. Four weeks after transplanting, the mean concentration of N in tomato transplants decreased with increase in the shoot residue concentrations. However, no significant difference was recorded between treatments B and C for N content (Table 5.6.1). The amount of P in treatments B, C and D and the mean concentration of K in the control and tomato transplants under the shoot residue treatment groups were not significantly different from one another. Although, the Mg and Na content in treatments B, C and D decreased with increase in residue concentrations, there was no significant difference in the amount of Na detected in treatments B and C.

The addition of the dried shoot residue of *A. arctotoides* significantly reduced the mean concentrations of the two micro nutrients (Cu and Fe) in the leaves of tomato transplants under the treatments at 4 weeks after transplanting (Table 5.6.1). Conversely, no significant variation was observed in the amount of Cu between treatments B and C. Interestingly, application of the two highest dried shoot residue concentrations had no effect on the uptake Zn by tomato leaves in treatments C and D. The mean concentrations of the nutrient (Zn) in both the control and treatment B was also similar but significantly lower than treatments C or D.

The data on the mineral analysis at 12 weeks after transplanting further revealed that the mean concentration of N in the leaves of tomato was similar to four weeks after transplanting. While the amount of P in the control and residue treated groups was not significantly different, the mean concentrations of K, Mg and Na varied considerably (Table 5.6.1). Statistically, no significant difference was recorded in the amount of K in the control and treatment B, the mean concentration of same nutrient in treatments C and D also followed similar trend. On the other hand, the addition of the shoot residue improved the Mg content of treatments B, C and D in comparison with the control. The amount of Na in the leaves of the transplants under treatment B was significantly higher and similar to the control.

Moreover, the mean concentrations of Na found in treatments C and D also followed similar trend but significantly lower than both the control and treatment B values. The results of the micro nutrients content indicated that the mean concentrations of Cu, Fe and Zn were maximum in the leaves of tomato transplants grown in soils with the highest residue concentration (treatment D). The amount of Cu detected in treatment C was also higher and similar to treatment D.

Table 5.6.1: Effects of the dried shoot residue of *A. arctotoides* on macro and micro nutrients in tomato leaves at 4 and 12 weeks after transplanting

Weeks after transplanting	Treatments	Macro nutrients (mg g^{-1})					Micro nutrients (mg g^{-1})		
		N	P	K	Mg	Na	Cu	Fe	Zn
4	A	1.69±0.09^{a}	17.10±1.54^{a}	12.39±1.25^{a}	0.93±0.08^{a}	1.65±0.15^{a}	2.14±0,24^{a}	11.86±1.17^{a}	52.58±1.72^{b}
	B	1.16±0.05^{b}	15.43±1.17^{b}	12.00±1.20^{a}	0.78±0.06^{b}	1.58±0.13^{ab}	1.96±0.25^{b}	7.85±0.82^{b}	52.91±1.69^{b}
	C	1.34±0.05^{b}	14.89±1.13^{b}	11.83±1.04^{a}	0.58±0.04^{c}	1.55±0.16^{b}	1.86±0.19^{b}	6.69±0.59^{c}	58.66±1.98^{a}
	D	1.08±0.02^{c}	14.73±1.08^{b}	11.52±0.92^{a}	0.49±0.05^{d}	1.47±0.12^{c}	1.72±0.21^{c}	3.56±0.45^{d}	61.03±2.14^{a}
12	A	0.73±0.08^{a}	13.05±1.31^{a}	13.65±1.10^{a}	1.55±0.17^{b}	1.56±0.20^{a}	34.42±2.53^{c}	195.6±4.33^{b}	16.62±1.11^{c}
	B	0.57±0.05^{b}	12.95±1.30^{a}	12.47±1.08^{ab}	1.86±0.22^{a}	1.53±0.18^{a}	37.99±2.28^{b}	211.2±3.89^{b}	18.40±1.17^{bc}
	C	0.61±0.05^{b}	12.48±1.18^{a}	10.81±0.93^{c}	1.77±0.25^{a}	0.62±0.12^{b}	43.87±3.04^{a}	211.6±4.27^{b}	19.62±1.11^{b}
	D	0.47±0.03^{c}	11.49±1.13^{a}	11.76±1.16^{b}	1.70±0.19^{a}	0.78±0.15^{b}	48.34±3.19^{a}	238.1±5.08^{a}	24.56±1.21^{a}

Data are means (± S.D). N= 4; Means followed by the same letter along the column are not significantly different at P > 0.05

Key: A = Control; B = 10 g; C = 20 g; D = 40 g residue

Effects of the dried shoot residue on mineral uptake by the leaves of cabbage at 4 and 12 weeks after transplanting

The trend of the mineral uptake by cabbage leaves at 4 weeks after transplanting was similar to those of tomatoes. During the period, there was no significant difference in the amount of P detected in the control and residue treated groups. However, the mean concentrations of N, K and Na consistently decreased with increase in the amount of the residue added to the soil (Table 5.6.1). The results further showed that the amount of K in treatment B was significantly higher but not significantly different from the control. The uptake of Na by the leaves of the two set of groups (A and B) also followed similar trend. While the amount of Mg in the control and cabbage under treatment B was the least, the Na content of the same treatment group was significantly higher. The uptake of Na by treatments C and D also followed similar trend but the two groups has the least amount of the mineral. At 4 weeks after transplanting, the mean concentrations of Cu and Fe were consistently lower in the control transplants than those grown with the dried shoot residue of *A. arctotoides* at all concentrations. However, the amount of Cu in treatments C and D plus the mean concentration of Fe in treatment groups B, C and D were all similar. When compared with the control, the amount of Zn detected in the leaves of cabbages under the shoot residue amended soils, decreased with increase in treatment concentrations (Table 5.6.2).

At 12 weeks after transplanting, the uptake of all nutrients with the exception of Mg and Zn by the leaves of cabbage transplants was not affected by the application of the shoot residue. During the period, the N and Na contents in treatment B were maximum whereas the least amount of the

two mineral nutrients was recorded in the control. Moreover, the mean concentrations of P and K in all the leave of transplants grown in each of the three residue treated soils were not significantly different in comparison with the control (treatment A). The amount of Mg and Zn in transplants under treatments B, C and D decreased consistently with increase in the concentrations of the residue (Table 5.6.2). However, the mean concentration of Zn obtained from the two treatment groups was not significantly different but significantly lower than the control. The results further indicated that the amount of Cu and Fe in treatments D was significantly higher than either of the other treatments including the control. The effects of the shoot residue on the mean concentration of Cu in treatments C and D and the Fe content of treatments B and C, all followed similar trend.

Effects of the dried shoot residue on soil residual minerals (mg g^{-1}) at 12 weeks after transplanting

The mean values of the residual mineral nutrients of soils with the two vegetable plants at 12 weeks after transplanting are presented in Table 5.6.3. The data indicated that the dried shoot residue of *A. arctotoides* considerably reduced the mean concentrations of N, P, K and Zn in the soils with tomato plants in comparison with the control. However, no significant difference was recorded for the N content among treatments B, C and D. The amount of P extracted from treatments B and C was not significantly different from the control. Statistically, the P content of

treatment D was not significantly different from treatment C but significantly lower than the control (A). The mean concentrations of K between the control and treatment B also followed similar trend with P content. Conversely, the amount of K in treatments C and D differed significantly.

Table 5.6.2: Effects of the dried shoot residue of *A. arctotoides* on macro and micro nutrients in cabbage leaves at 4 and 12 weeks after transplanting

Weeks after transplanting	Treatments	Macro nutrients (mg g^{-1})					Micro nutrients (mg g^{-1})		
		N	P	K	Mg	Na	Cu	Fe	Zn
4	A	0.68 ± 0.05^{a}	14.75 ± 1.12^{a}	12.11 ± 1.10^{a}	0.86 ± 0.11^{c}	2.42 ± 0.23^{a}	6.22 ± 0.64^{c}	21.79 ± 1.24^{b}	31.64 ± 1.30^{a}
	B	0.51 ± 0.03^{b}	14.34 ± 1.08^{a}	12.12 ± 1.08^{a}	0.88 ± 0.15^{c}	2.59 ± 0.24^{a}	7.76 ± 0.78^{a}	33.93 ± 2.13^{a}	26.60 ± 2.24^{b}
	C	0.26 ± 0.02^{c}	13.45 ± 1.05^{a}	11.06 ± 1.11^{b}	0.95 ± 0.16^{b}	2.11 ± 0.20^{b}	6.62 ± 0.65^{b}	32.67 ± 2.20^{a}	22.96 ± 1.26^{c}
	D	0.27 ± 0.02^{c}	14.19 ± 1.11^{a}	8.36 ± 0.92^{c}	1.07 ± 0.13^{a}	1.94 ± 0.16^{b}	6.57 ± 0.60^{bc}	33.38 ± 2.25^{a}	19.80 ± 1.18^{d}
12	A	0.54 ± 0.03^{c}	9.72 ± 0.82^{b}	8.08 ± 0.55^{b}	1.53 ± 0.04^{a}	2.07 ± 0.18^{d}	23.69 ± 1.32^{c}	38.45 ± 2.44^{c}	13.14 ± 1.15^{a}
	B	0.84 ± 0.06^{a}	11.87 ± 1.11^{a}	9.81 ± 0.78^{a}	1.00 ± 0.02^{b}	1.81 ± 0.32^{a}	41.62 ± 2.30^{b}	59.33 ± 2.61^{b}	11.51 ± 1.11^{ab}
	C	0.67 ± 0.05^{b}	11.04 ± 1.05^{a}	9.74 ± 0.84^{a}	0.89 ± 0.03^{c}	2.68 ± 0.29^{b}	44.37 ± 2.27^{b}	64.16 ± 2.80^{b}	10.57 ± 1.09^{b}
	D	0.71 ± 0.05^{b}	11.64 ± 1.15^{a}	10.55 ± 1.03^{a}	0.79 ± 0.02^{d}	2.41 ± 0.25^{c}	49.27 ± 2.54^{a}	77.35 ± 3.07^{a}	10.05 ± 0.78^{b}

Data are means (± S.D). N=4; Means followed by the same letter along the column are not significantly different at P > 0.05

Key: A = Control; B = 10 g; C = 20 g; D = 40 g residue

Interestingly, the residual Fe content of soils with tomato amended with residue treatments C and D was not significantly ($P > 0.05$) different and significantly higher than the least treated soil. The control had the lowest amount of mineral iron (Fe). In the soils with cabbage transplants, the results showed that the mean concentrations of N, P and Zn in the control and treatments B, C and D were not significantly different after 12 weeks of transplanting (Table 5.6.3). However, the quantities of K and Fe varied considerably among all the groups. While no significant difference was recorded in the K content of the control and soils under treatments B and D, the mean concentration of Fe in treatments C and D was significantly higher and similar than the control and treatment B.

Table 5.6.3A: Effects of the dried shoot residue of *A. arctotoides* on tomato residual soil nutrients (mg g^{-1}) at 12 weeks after transplanting

Treatments	Tomato soil residual nutrients (mg g^{-1})				
	N	P	K	Fe	Zn
A	0.12±0.01[a]	9.44±0.50[a]	1.85±0.24[a]	98.4±3.42[c]	32.70±1.66[a]
B	0.09±0.01[b]	10.07±0.44[a]	1.74±0.17[a]	141.6±3.56[b]	25.64±1.34[b]
C	0.07±0.01[b]	8.87±0.35[ab]	1.57±0.12[b]	215.8±4.38[a]	18.46±1.28[c]
D	0.09±0.02[b]	7.73±0.40[b]	1.33±0.08[c]	217.2±4.51[a]	14.90±1.31[d]

Data are means (± S.D). N=4; Means followed by the same letter along the column are not significantly different at $P > 0.05$

Key: A = Control; B = 10 g; C = 20 g; D = 40 g residue

Table 5.6.3B: Effects of the dried shoot residue of *A. arctotoides* on cabbage soil residual nutrients (mg g^{-1}) at 12 weeks after transplanting

Treatments	Cabbage soil residual nutrients (mg g^{-1})				
	N	P	K	Fe	Zn
A	0.13±0.01[a]	10.91±1.08[a]	1.85±0.27[a]	89.26±2.74[b]	18.38±1.16[a]
B	0.12±0.01[a]	11.81±1.13[a]	1.44±0.18[b]	90.02±2.68[b]	16.22±1.11[a]
C	0.09±0.02[a]	11.54±1.20[a]	1.04±0.13[c]	309.19±3.97[a]	16.88±1.14[a]
D	0.14±0.03[a]	12.44±1.25[a]	1.61±0.30[ab]	302.40±4.01[a]	18.66±1.23[a]

Data are means (± S.D). N=4; Means followed by the same letter along the column are not significantly different at $P > 0.05$

Key: A = Control; B = 10 g; C = 20 g; D = 40 g residue

CHAPTER SIX

DISCUSSION

CHAPTER SIX

6.0 DISCUSSION

6.1 The foliar morphology of *A. arctotoides* using the Scanning Electron Microscope

The authentication of microscopically important ultra structures using the Scanning Electron Microscope (SEM) plays a vital role in the identification, location and distribution of some botanical features of plants (Vaishali et al., 2008). Functionally, the forms and sizes of different types of trichomes have been linked to the accumulation and secretion of organic metabolites which usually appear as crystal deposits on the epidermal layers of studied tissues. In this study, the presence of anisocytic stomata, capitate glandular trichomes (GTs) and filamentous non glandular trichomes (NGTs) revealed by the SEM were the three major ultra structures on the upper (adaxial) and lower (abaxial) leaf surfaces of *A. arctotoides.* These observations support similar findings earlier reported by Adedeji and Jewola (2004) from a comparative study of several genera of the Asteraceae family.

The detection of Na^{+}, Mg^{2+} and Ca^{2+} as the ionic constituents of the crystal around the stomata and GTs of *A. arctotoides* corroborates Turner et al. (2000) who indicated that GTs are organs of secretion of natural products. Ashafa et al. (2008) also reported similar observations from related studies on *Felicia muricata,* a medicinal species of the *Asteraceae* family. Economically, several classes of secondary metabolites sequestered

from the GTs of many plants have been associated with phytotoxicity on seed germination and seedling growth or other plants. The organic compounds such as phenolics and sesquiterpene earlier reported by the authors (Oyedeji et al., 2005; Sultana and Afolayan, 2007) from the different parts of *A. arctotoides* might be secreted products of the GTs of the plant. The inhibitory and herbivory potentials of such compounds of both the GTs and NGTs against other vegetations as well as insect and airborne propagules of fungi have been confirmed (Afolayan and Mayer, 1995). The occurrence of the two types of trichommes on the leaf surfaces of *A. arctotoides* and secretion of ionic compounds might be responsible for the dominant growth habit. Other functions such as regulation of heat stress / freezing and ultra violet light performed by the NGTs might also account for the ability of *A. arctotoides* to survive the extreme weather conditions of the Eastern Cape Province of South Africa.

6.2 Phytochemical composition of the root and shoot extracts of *A. arctotoides*

The production of a wide range of natural products by plants and the importance of phytochemical compounds as sources of therapeutic and industrial raw materials have been well documented (Viles and Reese, 1996). From the present study, the observed variations in the chemical compositions of the root and shoot materials of *A. arctotoides* implied that the aerial part of the plant contained more bioactive constituents than the underground portion. This result validates the findings of Parvez et al. (2004) who

reported differences in the chemical compositions of the organic constituents in tissues and organs of the donor plants. The absence of cardiac glycosides and steroids in the root material of *A. arctotoides* during this study corroborates the above statement. The results of the quantitative estimates of the percentage crude yields further showed some variations between the two plant parts of *A. arctotoides.* While higher percentage of flavonoids was recorded in the shoot material, the root sample on the other hand contained more of alkaloids. The percentages of phenols and tannins obtained from both the root and shoot were quiet low, however, the shoot had more of the compounds than the root. Although, many of these organic substances are noted for curative and pesticidal activities, the bioactive constituents conferring these two attributes on natural products of plant origin have also been implicated in phytotoxicity on seed germination and seedling development of other plants (Hassan and Ghareib, 2009).

The inhibitory activities of phytochemicals such as *p* –coumaric, and ferulic acids (derivatives of phenolic) sesquiterpene lactones and volatile constituents of essential oils from plants, particularly the *Asteraceae* family have been reported (Vyvyan, 2002). For instance, the negative impact of *p* –coumaric, alkaloids, flavonoids and phenolic acids on plant water utilization, leaf area expansion of many crops and soybean root lignifications caused by ferulic acids have been confirmed (Blum and Gerig, 2006). Similarly, María and Julio (2004) also reported the oxidative stress caused by 1,8 cineole, thymol, geraniol, menthol and camphor isolated from plants on maize (*Zea mays* L.) root growth. Thus, the quantitative estimates of cardiac glycosides, saponins, steroids and triterpenes were not determined in the present study because there is dearth of information on the allelopathic activities of these organic substances.

6.3 Allelopathic effects of root and shoot aqueous extracts of *A. arctotoides* on seed germination and seedling growth of selected vegetables

The high percentage viabilities recorded for the seeds of cabbage, carrot, tomato and spinach indicated that all the seeds were ideal for the study. The results also provided a good platform for assessing the allelopathic effects of the root and shoot aqueous extracts of *A. arctotoides* on the four vegetable seeds.

Generally, the mean percentage inhibitions on the germination, radicle and plumule elongations of cabbage, carrot, tomato and spinach seeds incubated with the shoot extract concentrations of *A. arctotoides* was significantly higher than the root. The responses of the four vegetables to the two extract treatments were however, concentration-dependent rather than the specific plant parts. Inderjit and Dakshini (1995) found that water extracts from several species of the Asteraceae family inhibited the germination and growth of other plant species. Therefore, the observed significant reductions in germination of the four vegetable seeds exhibited by the root and shoot aqueous extracts of *A. arctotoides* in this present study were consistent with early reports (Inderjit and Dakshini, 1995).

Species sensitivity and tolerance to different allelochemicals are important indices of dose-dependent relationships characterized by both stimulatory and inhibitory reactions at the varying extract concentrations (Calabrese et al., 2005). The 100% germination inhibition recorded for cabbage and carrot seeds treated with the shoot extracts at 10

mg/ml support (Calabrese et al., 2005). The observed insignificant treatment effects caused by the root extracts at 2 and 4 mg/ml on carrot, tomato and spinach seed germination also agrees with earlier findings of Nazir et al. (2007) who reported similar observations on some agricultural crops treated with medicinal plant extracts. Although, the effects of the treatment on radicle and plumule growth of the vegetables followed similar trend with that of seed germination, the radicle growths of the four crops evaluated in the present study were more affected than the plumule. Leather and Einhellig (1985) indicated that an extract concentration of as low as $1/25^{th}$ of a mg was sufficient to completely reduce radicle growth susceptible target. The observed drastic reductions of about 89.7% and 94.1% on the radicle length relative to 72.3% and 83.5% recorded for the plumule growth of tomato and spinach treated with the shoot extract at 10 ml/mg respectively agree with Leather and Einhellig (1985). Similar trend was also noticed with the root extract at the same concentration but with lesser percentage inhibitions. The higher reduction values obtained from radicle length might be attributed to direct contact of the organ with the water soluble allelochemicals which were not readily translocated to the plumule.

Seed germination and seedling development of different crop species have been attributed to a number of factors. At higher extract concentrations, increase in electrolyte leakages and loss of membrane integrity lead to lipid peroxidation (Bogatek et al., 2006). Alterations in the enzymatic activities have also been linked to the immobilization of reserve compounds during germination. These processes might invariably reduce or completely inhibit seed germinability as observed in cabbage and carrot seeds in the present study. Hegazy and Fadl-Allah (1995) also reported that

increase in the susceptibility of seedlings exposed to allelochemicals lead to some morphological abnormalities. Interestingly, the observed brownish colouration and increased thickness in the radicles of the two vegetables incubated with 8 and 10 mg/ml of the root and shoot extracts of *A. arctotoides* supported the earlier findings (Hegazy and Fadl-Allah 1995).

6.4 Allelopathic effect of the dried shoot residue of *A. arctotoides* on the morphology, growth and chlorophyll accumulation of tomato and cabbage

In this study, the general observations, such as chlorosis and necrotic lesions on the leaf surfaces of the two vegetables under treatments B, C and D were more obvious at 1 and 2 weeks after transplanting than at any other sampling period. However, at 3 and 4 weeks after transplanting, considerable increases were recorded in the number of leaves, leaf area, fresh and dry shoot weight among all the groups due to new growth. In a related study, Pandey et al. (1993) attributed wilting of water hyacinth leaves to the presence of growth inhibitors in *Parthenin hysterophorus* L. dried leaf residues. El-Khatib et al. (2004) further asserted that reductions in water and nutrient absorption by plant roots appear to be direct inhibitory effects of allelochemicals in the residue. Thus, the wide variations and significant differences in the number of leaves, leaf area, shoot dry weight and root morphology as well as yield and yield components between the control and groups B, C and D might be attributed to the presence of phytotoxic

substances in *A. arctotoides* shoot residue as reported by previous authors (Pandey et al. 1993; El-Khatib et al. 2004).

Generally, the inhibition percentages in the number of leaves, leaf area, fresh and dry shoot weight were concentration-dependent. Dose-dependent relationships have been well documented by many workers. El-Khatib et al. (2004) reported an inverse correlation between *Chenopodium murale* shoot extracts and leaf area of some crops. Also, Morales-Payan et al. (1998) observed similar effects on the purple nutsedge density on dry shoot and root weights of radish and bell pepper. Thus, the observed differences in the number of leaves, leaf area, dry root and shoot weight as well as root length and area between the control and shoot residue treated transplants support the above findings. When compared with the control, the losses in dry shoot weight of tomato under treatments B, C and D at 4 weeks after transplanting were 38.6, 45.5 and 70.3% respectively. Cabbage transplants under the same treatments were also reduced by 57.5, 73.3 and 87.5%. Likewise, the declines in the dry root weight of the two crops during the same period were 61.3, 82.9 and 83.4% plus 53.1, 54.7 and 67.2% for treatments B, C and D respectively. The significant differences between the inhibition percentages in the dry shoot and root weights were indicative of differential sensitivity of tomato and cabbage transplants used in this study. While the dry shoot weight of cabbage was more inhibited by treatment effects, the dry root weight of tomato appeared to be drastically suppressed even though, no significant difference was observed between treatments C and D.

The data on chlorophyll *a* and *b* pigments accumulation showed that there was no regular pattern in the accumulation of chlorophyll *a* and *b* pigments in the leaves of the vegetables grown in the amended soils. Einhellig et al. (1993) stated that allelochemicals cause significant reductions in chlorophyll contents of the test plants. The results from the present study were not consistent with this study. However, the observed insignificant differences in chlorophyll *a* and *b* pigment contents between the control and treatment B relative to the others support the above statement.

6.5 Allelopathic effects of the dried shoot residue of *A. arctotoides* on yield and yield components of tomato and cabbage at 12 weeks after transplanting

The total fruit yield and shoot dry weight of all tomato groups B, C and D decreased with increase in treatment concentrations. Similar observation was recorded in the fresh head weight of cabbages but there were no differences in the dry head weight of the treatments in comparison with the control. The extent of inhibitory effects of allelochemicals on crop yields are concentration related Weidenhamer et al. (1989). Thus, the significant reductions due to addition of the shoot residue at the two highest concentrations (treatments C and D) of between 37.2 to 84.8% in tomato fruit yields and 30.9 to 72.4% recorded for fresh head weight of cabbage agree with Weidenhamer et al. (1989).

6.6 Allelopathic effects of the dried shoot residue of *A. arctotoides* on mineral uptake by the leaves of the two vegetables and soil residual nutrient content

The uptake of certain mineral by the two vegetable leaves particularly among cabbage transplants was generally not consistent with treatment concentrations. The mean concentrations of N, K, Na and Zn in the leaves of tomato and cabbage treatments B, C and D decreased with increase in the amount residue added to the soil. It has been well documented that plant roots compete for mobile nutrients such as sulphates or nitrates than they do for P, Zn and perhaps K in the soil (Nye et al., 1977). Weir et al. (2004) also reported that ferulic acid is an abundant allelochemicals in the soil which affect root morphology, biomass partitioning and reserve allocation. These authors further asserted that plant roots exposed to ferulic acid showed reduction in water and mineral utilization which eventually lead to decrease in foliar expansion and root elongation. Thus, the observed variations in the mean concentrations of nutrients in the leaves of the two vegetables might be attributed to inability of the root systems of the affected vegetables to effectively transport the essential nutrients from the potting medium to the aerial parts of the plant.

Differences in the concentrations of allelochemicals in the soil have been linked to factors such as clay mineral and chemical transformation by microorganisms (Blum et al., 1999). These invariably affect rates of photosynthesis and nutrient uptake by the plant roots. The differences in the uptake of nutrients by the leaves support the findings of Alam and Islam (2002). The authors observed variations in the nutrient contents of the shoot of several plant species. The availability of one or more mineral nutrients in

plant tissues due to the presence of another has been debated by many workers. Mersie and Singh (1988) observed variations in K, Ca, Mg, Fe and Zn contents in tomato plants treated with several classes of phenolic acids. IRRI (1979) reported the effect of Na on the uptake of K. Thus, the differences in the uptake of some minerals by treatments B, C and D relative to the control in this study might be attributed partially to the inhibitory effect of allelochemicals released by *A. arctotoides* dried shoot residue. Excessive release of antagonistic minerals might also affect the uptake of the minerals under review.

The decreases in soil residual mineral elements might be attributed to the rates of soil mineralization intensified by variations in the rates of residue decomposition and nutrient release (Tian, 1992). Blum et al. (1999) have also suggested the impacts of chemical transformation by microorganisms on soil residual nutrients.

CHAPTER SEVEN

SUMMARY AND CONCLUSION

CHAPTER SEVEN

7.0 SUMMARY AND CONCLUSION

The integration of two or more plants into the traditional farming systems is a laudable practice that enriches soil fertility and soil organic matter. However, through this same practice, a number of plants also produce and release several classes of phytochemicals that adversely affect the growth of other vegetations. In South Africa, the awareness of the demand for herbal remedies and high unemployment rate have led to over-exploitation of many indigenous species. Thus, the fear of near-extinction of valuable medicinal herbs has motivated research towards the establishment of scientific evidence of allelopathic interactions among such plants being integrated into the farming systems. Hence, the selection and need for systematic and scientific investigations of the allelopathic effects of the shoot and root of *Arctotis arctotoides* (L.f). O. Hoffm on some vegetables.

The results of the current study have demonstrated that *A. arctotoides* possess some ultra structures which might functionally be responsible for the production and storage of some phytochemicals including; alkaloids, flavonoids, phenols, steroids and tannins, identified from the root and shoot of the plant. The results further showed that both the root and shoot aqueous extracts of the plants exhibited significant inhibitory effects on the germination, radicle and plumule length of cabbage, carrot, tomato and spinach evaluated in this study. However, shoot extracts at 10 mg/ml had 100% inhibition on cabbage and carrot seed germination. The radicle and plumule length of the four

vegetables were also severely inhibited by the extracts. Generally, the trend of their sensitivity was in the order of cabbage > carrot > tomato > spinach.

When compared with treatment A (control), addition of the shoot residue drastically reduced the number of leaves, leaf area, dry shoot and root weights of tomato and cabbage transplants during the different growth stages. At 4 weeks after transplanting, the losses in the dry root weights of treatments B, C and D were 61.3, 82.9 and 83.4% for tomato and 53.1, 54.7 and 67.2% for cabbage respectively. The accumulation of chlorophyll *a* and *b* in the leaves of both vegetables varied among the treatments. Most often, the mean concentrations of the two pigments in the control and those grown with the least residue were not significantly different at 2 and 3 weeks after transplanting. Similar treatment effects were noticed with the nutrient uptake by the vegetable leaves but the shoot residue added to the soil greatly improved the uptake of micro nutrients by cabbage leaves at 12 weeks after transplanting.

Finally, the results from the present study have demonstrated a clear evidence of allelopathic effects of the root and shoot of *A. arctotoides* under laboratory and greenhouse conditions. Therefore, mulching of *A. arctotoides* shoot residue into the soil at higher concentrations might have adverse effects on economic crops. However, the impacts of microbial population which were not considered in this study might be a gap for future research.

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APPENDICES

Appendix I: Tomato transplants at 4 weeks after transplanting

Key: A = Control; B = 10 g; C = 20 g; D = 40 g residue

Appendix 2: Cabbage transplants at 4 weeks after transplanting

Key: A = Control; B = 10 g; C = 20 g; D = 40 g residue

Appendix 3: The Foliar Micromorphology of *Arctotis arctotoides* using Scanning Electron Microscope (Published article).