Influence of abiotic stress on allelopathic properties of *Amaranthus cruentus* L.

by

INGRID ALLEMANN

Submitted in fulfilment of the requirements for the degree

Magister Scientiae

in the

Faculty of Natural and Agricultural Sciences

Department of Plant Sciences

University of the Free State

Bloemfontein

JUNE 2016

Supervisor:

Dr M.E. Cawood

Department of Plant Sciences

UFS

Co-supervisor:

Dr J. Allemann

Department of Soil, Crop and Climate Sciences

UFS

Declaration

I declare that the dissertation hereby submitted by me for the MSc degree at the University of the Free State is my own independent work and has not previously been submitted by me at another university/faculty.

I further more cede copyright of the dissertation in favour of the University of the Free State.

Acknowledgements

- I would like to thank the Dean's office (Faculty of Natural and Agricultural Sciences) for funding to help me complete my studies.
- I am sincerely indebted to Dr M.E. Cawood and Dr J. Allemann for their supervision, guidance, patience and helpful criticism throughout my study.
- My gratitude also goes to the Department of Agriculture, University of the Free State, South Africa, for providing the climate controlled chambers and help with getting soil that was used in this study.
- I thank Prof. W. Swart, Plant Pathology, for allowing me to use part of his laboratory for my experiments.
- I thank Dr A. Biljon, Plant Breeding, for giving me a helping hand with understanding the HPLC.
- I am deeply grateful to my family members, especially my mom Anette and dad James, my two sisters Melanie and Elaine, my brother in law Jac, my Ouma and Oupa, Uncle, Aunt and two nieces, for their unconditional love and encouragement and always listening to me complain. I love you all.
- To all my friends in the department for always helping and lending a shoulder to cry on. All the best to you all.
- En die beste vir laaste Andri en Linde. My twee beste pelle in die wêreld. As dit nie vir julle was nie so ek nooit klaar gemaak het nie. Ek is baie lief vir julle.

Table of contents


Dr J Allemann

List of
Tables & Figures

List of Tables

List of Figures

CHAPTER 1

INTRODUCTION AND RATIONALE

1. Introduction and rationale

Some 4 000 edible plant species have been used by humans in the past as a source of food (Chweya, 1989). Over many years this number has decreased to concentrate on fewer and fewer plants. One hundred and fifty plant species are widely cultivated today, and of these only 20 make up most of the world's food supply (Chweya, 1989). Rice, wheat and maize provide approximately 60% of the food supply, making these three plant species vital for food security (Anon, 1993). Many edible plant species across the world have not yet been developed to their full food crop potential.

Since ancient times there have been a wide variety of indigenous and minor crops that have been utilized for daily consumption. Many of these plants are used as traditional food in order to maintain a healthy lifestyle (Kazuhiko *et al.,* 2002). When determining which plants are most advantageous for a healthy lifestyle, it is important to look at the plants' phytochemical constituents. A number of plant extracts have been tested for their bioactivity using many different *in vitro* modelling systems. Understanding the biological functions and active constituents of different plant species may help with the improvement of eating habits and public health in developing countries (Maiyo *et al.,* 2010). The poor nutritional values of the few, but most produced crop species in the world today and continuous erosion of cultivated land, are some of the reasons for renewed interest in alternative crops. The use of alternative crops would result in product competitiveness, rich nutritional value, tradition, locality and special quality (Bavec & Bavec, 2006). Kazuhiko *et al*. (2002) stated that the potential use and addition of such local agriculture products will increase and stabilise the income of farmers in rural areas.

Amaranth is one of the few multi-purpose crops which can supply grain as well as tasty leafy vegetables of high nutritional quality and function as a food and animal fodder (Tucker, 1986). Although the crop was one of the staple foods in the pre-colonized South American civilizations, the cultivation and knowledge fell into oblivion and thus nowadays it is classified as a new, forgotten, neglected and alternative crop of great nutritional value (Teutonico & Knorr, 1985). Amaranth plants contain sufficient amounts of important micro-nutrients including minerals and vitamins as well as an adequate amount of other bio-active components or health

protecting nutrients (nutraceuticals) as dietary supplements (Mensah *et al.*, 2008; Maiyo *et al.*, 2010; Nana *et al.*, 2012; Alemayehu *et al*., 2015). Due to its ability to adapt to a wide range of environments, amaranth has been considered as a promising crop for marginal lands and semiarid regions (Allemann *et al*., 1996). Researchers around the world have focused on improving the agronomic features of the plant which include nutritional quality and processing of the seeds (Yaacob *et al*., 2004; Radosavljević 2006).

Many species of weeds, as well as crop plants, are known to be allelopathic. Allelochemicals are secondary metabolites present as soluble or volatile compounds found in different plant organs, including leaves, flowers, fruits, and buds (Rice, 1984), which may substantially differ in allelopathic activity (Ciarka *et al*., 2009). The allelopathic potential of many plants is strengthened by exposure to various environmental stresses (Einhellig, 1987; 1996) and induces a variety of phytochemical compounds (Josep & Joan, 1997; Kong *et al*., 2000). These substances are released directly from the living plant into the surrounding environment through root exudation, leaching and volatilization, and when the plant dies and decomposes (Rice 1984; Weston 2005). Allelopathic compounds prevent or retard the germination and development of certain plants and this can lead to a decrease in overall yield, quality and also harvest efficiency of agricultural crops (Putnam 1986, Guo & Al-Khatib 2003). Allelopathy therefore, plays a role in many plant communities and has been reported in both sorghum and sunflower (Menges, 1987). The allelopathic effects of pigweed (*Amaranthus retroflexus* L.) are well documented (Qasem, 1995; Rezaie & Yarnia, 2009), while those of grain amaranth are not (Machado, 2007). Recently, it was found that aqueous extracts of tested grain amaranth exert allelopathic activity. Compared to the pigweed amaranth, the grain species displayed a stronger inhibitory effect on the germination process, and root elongation of garden cress (Mlakar *et al*., 2012).

These results point out the problematic consequence when amaranth is cultivated in crop rotation systems. Furthermore, climate change influences a plant's chemical response and the ecological function of plant allelochemicals (Harvey & Malcicka 2015). Sudden changes in temperature may influence the production of chemical compounds and the allelopathic properties of a plant. Therefore this study's aim was

to investigate the relationship between environmental variation and allelopathic effects of plant litter and extracts of *Amaranthus cruentus* on the germination and growth of some vegetable species in order to determine the crops' feasibility for intercropping and in a rotation system.

It is hypothesised that a change in temperature will have an influence on the secondary metabolites and therefore alters the allelopathy of *A. cruentus*.

Objectives:

1) Grow *A. cruentus* plants under optimal temperature conditions for 3 months, where after plants will be subjected to cold and hot temperature stress. Plants kept at optimal temperature conditions will serve as controls for temperature stress.

2) Determine total phenolic and flavonoid content in leaf litter of all the temperature treatments.

3) General identification of polar and non-polar compounds from the leaves of the different temperature treatments of *A. cruentus*, using thin layer chromatography with different detection sprays, high pressure liquid chromatography and gas chromatography coupled with mass spectroscopy.

4) Antioxidant and antibacterial activity of polar and non-polar extracts.

5) The *in vitro* phytotoxicity of leaf litter and extracts of *A. cruentus* on the percentage germination and organ length of various vegetable seeds, using the modified Sandwich method.

References

Alemayehu, F. R., Bendevis, M. A., Jacobsen, S.E. 2015. The Potential for Utilizing the Seed Crop Amaranth (*Amaranthus* spp.) in East Africa as an Alternative Crop to Support Food Security and Climate Change Mitigation. *J. Agron. Crop Sci*, **201**: 321–329. doi: 10.1111/jac.12108.

Allemann, J., van den Heever, E., Viljoen, J. 1996. Evaluation of Amaranthus as a possible vegetable crop. *Appl. Plant Sci*. **10**: 1-4.

Anon., 1993. Nurturing a cornucopia of potential. *Washington Post* 20 September.

Bavec, F., Bavec, M. 2006. Grain amaranths. In: Organic Production and Use of Alternative Crops. CRC Press/Taylor and Francis, Florida. Pp 88–98.

Chweya, J.A. 1989. The role of indigenous vegetation in human food production. A paper presented at the Indigenous Vegetation Experience Sharing Symposium. Organised by Kengo, 20-22 September 1989. JKCAT (Thika), Kenya.

Ciarka, D., Gawronska, H., Szawlowska, U., Gawronski, S.W. 2009. Allelopathic potential of sunflower. I. Effects of genotypes, organs and biomass partitioning. *Allelopathy J*. **23**: 95–109.

Einhellig, F. A. 1987. Interactions among allelochemicals and other stress factors of the plant environment. ACS Symp. Ser. **330**: 343–357.

Einhellig, F. A. 1996. Interactions involving allelopathy in cropping system. *Agron. J*. **88**: 886–893.

Guo, P., Al-Khatib, K. 2003. Temperature effects on germination and growth of redroot pigweed (*Amaranthus retroflexus*), Palmer amaranth (*A. plameri*), and common waterhemp (*A. rudis*). *Weed Sci*. **51**: 869-875.

Harvey, J.A., Malcicka, M. 2015. Climate Change, Range Shifts and Multitrophic Interactions. Chapter 4: Biodiversity in Ecosystems- Linking Structure and Function. Publisher: InTechOpen. Pp 85-109.

Josep, P., Joan, L. 1997. Effects of carbon dioxide, water supply and seasonally on terpene content and emission by Rosmarinus officinalis. *J. Chem. Ecol*. **23**: 979–993.

Kazuhiko, N., Molay, K.R., Najeeb, S.A., Vipaporn, N.T., Gassinee, G. 2002. Inventory of indigenous plants and minor crops in Thailand based on their bioactivity. *J. Agri*. *Prod*. **44**: 135-139.

Kong, C., XU, T., HU, F., Huang, S. 2000. Allelopathy under environmental stress and its induced mechanism. *Acta Ecol. Sin*. **20**: 849–854.

Machado, S. 2007. Allelopathic potential of various plant species on downy brome: implications for weed control in wheat production. *Agron. J*. **99:** 127–132.

Maiyo, Z.C., Ngure, R.M., Matasyoh, J.C., Chepkorir, R. 2010. Phytochemical constituents and antimicrobial activity of leaf extracts of three *Amaranthus* plant species. *Afri. J. Biotechnol.* **9**(21): 3178-3182.

Menges, R.M. 1987. Allelopathic Effects of Palmer Amaranth (*Amaranthus palmeri*) and Other Plant Residues in Soil. *Weed Sci*. **35**: 339-347.

Mensah, J.K., Okoli, R.I., Ohaju-Obodo, J.O., Eifediyi, K. 2008. Phytochemical, nutritional and medical properties of some leafy vegetables consumed by Edo people of Nigeria. *Afri. J. Biotechnol*. **7**(14): 2304-2309.

Mlakar, S.G., Jakop, M., Bavec, M., Bavec, F. 2012. Allelopathic effects of *Amaranthus retroflexus* and *Amaranthus cruentus* extracts on germination of garden cress. *Afri. J. Agri. Res*. **10**: 1492-1497.

Nana, F.W., Hilou, A., Millogo, J.F., Nacoulma, O.G. 2012. Phytochemical Composition, Antioxidant and Xanthine Oxidase Inhibitory Activities of *Amaranthus cruentus* L. and *Amaranthus hybridus* L. Extracts. *Pharmaceuticals*. **5**(6): 613-628.

Putnam, AR. 1986. The Science of allelopathy, John Wiley & Sons. New York.

Qasem, J. R. 1995. The allelopathic effect of three *Amaranthus* spp. (pigweeds) on wheat (*Triticum durum*). *Weed Res*. **35**: 41–49.

Radosavljević, M. 2006. Comparison of *Amaranthus cruentus* and *Zea mays*, L. starch characteristics. *Genetika*. **38**: 31-36.

Rezaie, F., Yarnia, M. 2009. Allelopathic effects of *Chenopodium album*, *Amaranthus retroflexus* and *Cynodon dactylon* on germination and growth of safflower. *J*. *Food Agri*. *Environ*. **7**(2): 516-521.

Rice, EL. 1984. Allelopathy. 2nd. Edn. Orlando, Florida, USA: Academic Press. Pp 424.

Teutonico, R.A., Knorr, D. 1985. Amaranth: Composition, properties, and applications of a rediscovered food crop. *Food Technol*. **39**: 49-60.

Tucker, J. B. 1986. Amaranth: the once and future crop. *Biosci*. **36**: 9-13. 59-60.

Weston, LA. 2005. History and Current trends in the use of allelopathy for weed management. *Hort Technol*. **15**(3): 529-534.

Yaacob, J.S., Taha, R.M., Mat Nor, N.A., Aziz, N. 2004. Pigment analysis and tissue culture of *Amaranthus cruentus* L. First International Symposium on Sustainable Vegetable Production in South East Asia. Eds.: De Neve, S., Boehme, M., Everaarts, A., Neeteson, J. *Acta Hort*. 958, ISHS 2012.

CHAPTER 2

LITERATURE REVIEW

2.1 *Amaranthus*

2.1.1 General

The Amaranthaceae family, also referred to as the Amaranth family, is part of the order Caryophyllales (Teutonico & Knorr 1985; Omosun *et al.*, 2008; Department of Agriculture, Forestry and Fisheries, 2010 (DAFF)). The plant species in the Amaranth family are vascular, flowering dicots that reproduce by seed. The plants are hardy, weedy, herbaceous, fast-growing, cereal-like plants. Mature amaranth plants are described as bushy with thick stalks; plants are erect or spreading and appear to be rough or prickly. Members of this family have simple leaves, which are arranged oppositely or alternately with the margins entirely or coarsely toothed and the plants lack stipules. The colour of the flowers, leaves and stems of grain amaranth vary but maroon or crimson is most common. Flowers are solitary or aggregated in cymes, spikes or panicles (Coastea & Demason, 2001; Hoiberg, 2016).

The Amaranth family contains approximately 23 genera and one of those is the genus *Amaranthus* (USDA, 2015). The genus *Amaranthus* consists of 70 species (Kachiguma *et al.,* 2015) and is divided into two subgenera, *Acnida* and *Amaranthus*. The sub-genus, *Acnida*, was formerly a genus of its own before being combined with genus *Amaranthus* and is made up of a group of 10 species (Steckel, 2007)*. Acnida* species are dioecious; therefore, the male and female flowers exist on separate plants. *Amaranthus* species are monoecious, so the male and female flowers are on the same plant (Mosyakin & Robertson, 1996). While floral distribution may vary across *Amaranthus* species, their geographical origins are similar.

From the 70 species, several are cultivated as leaf vegetables, grains or ornamental plants, while others are weeds. The heights of plants vary between 0.3 m and 2 m, which is dependent on the species, habitat and growing environment (DAFF, 2010). The Amaranthaceae family can have a seed yield of up to 3 t ha^{-1} when it is grown in monoculture for 3-4 months, while leaf yield can be 4.5 t DM ha^{-1} after 4 weeks (Teutonico & Knorr, 1985). Amaranth is a cosmopolitan genus of annual or short-lived perennial plants that grow in summer or autumn (Graber, 2014). Teutonico and Knorr (1985) referred to amaranth “as an under exploited plant with promising

economic value” that has only recently become recognized by the National Academy of Sciences.

2.1.2 Origin and history

Amaranth has a colourful history, is highly nutritious and the plant itself is extremely attractive and useful. Before the arrival of the European conquerors (Spaniards), the empire of the Aztec in ancient Mexico had developed a remarkable and advanced civilization. One of the cornerstones of this civilization was a tiny grain seed called amaranth. The name amaranth comes from the Greek amarantos which means, "One that does not wither", or “the never-fading" according to Graber (2014). This description refers to the flowers of the amaranth plant.

Amaranthus has no specific life expectancy as seed unearthed from ancient ruins will still sprout today. Amaranth is known to exist for more than 5500 years ago and the first recorded archaeological find was in Tehuacan Puebla, Mexico at about 4000 B.C. (Teutonico & Knorr, 1985). It was a very important crop during Aztec Empire and played a double role: First, it formed the basis of the Aztec diet, it was their largest land crop and was noted to be nourishes to infants and was used to provide energy and strength to soldiers on extended trips (Bermejo & Leon, 1994). This makes amaranth one of the oldest known food crops. Secondly, amaranth was involved in the Aztec ceremonial culture where images of their gods were made from a mixture of amaranth and honey (Ruskin, 1984).

After the Spanish defeated the Aztecs in 1519, they realized the importance of amaranth to the Aztec culture and set out to destroy it, hence fields of amaranth were burned and farmers found growing it, punished (Ruskin, 1984). Amaranth was replaced with corn and beans, though it was never eradicated as farmers in the remote parts of Mexico continued to grow it (Ruskin, 1984; Tucker, 1986). By the 17th century, the plant had spread through European gardens as an ornamental (Saunders & Becker, 1984) and was grown as a minor grain crop in Central Europe and Russia and eaten as mush and groats (Saunders & Becker, 1984). It spread to India and Ceylon in the 18th century and by the early 19th century, it had been taken to Africa and Asia where it is now planted as a grain crop in such widely scattered

regions as the Mountains of Ethiopia, the hills of South India, Nepal, Himalaya and plains of Mongolia (Becker *et al*., 1981).

Grain amaranth (*Amaranthus* species) is known as a pseudo-cereal and both the grain and leaves are used as a food source in southern Africa (Grosz-Heilman *et al*., 1990; Myers, 1996; Yangzhou & Stuttgart 1999). The leaves are often cooked fresh or sun-dried and stored for winter use (Stallknecht & Schulz-Schaeffer 1993; Shukla *et al*., 2005; Graber 2014). It was introduced to Kenya in 1983 and the Kenyan government recognized amaranth and formally announced it as a crop on the 5th July 1991 (Mwangi, 1993). Since its introduction and recognition in Kenya, the government has helped promote amaranth especially in semi-arid areas where other cash crops are not available. Amaranth is not usually planted in South Africa but occurs as a volunteer crop after the first rains and is harvested from the wild. The main reason for cultivation of this plant in South Africa is for household food security and replenishment of the seed bank. The main producing areas of amaranth in South Africa are Limpopo, North West, Mpumalanga and KwaZulu-Natal provinces. The cultivars planted are: *Amaranthus cruentus, A. hybridus, A. spinosus, A. caudatus, A. thunbergii*, which are all indigenous to the country (DAFF, 2010). Other species of amaranth are as shown in table 2.1.

Table 2.1: Species of amaranth, their origin and use.

Species	**Source**	**Use**	**Areas of origin**
A. lividus	Cultivated	Vegetable, ornamental	Asia
A. caudatus (A. edulis)	Cultivated	Grain, vegetable, ornamental	South America
A. cruentus (A. paniculatus)	Weed, cultivated	Grain, vegetable	South America
A. dubius	Weed, cultivated	Vegetable	South America
A. hybridus	Cultivated	Grain, vegetable	South America
A. hypochondriacus	Cultivated	Vegetable, grain	North America
A. retroflexus	Weed	Vegetable	North America
A. spinosus	Weed	Vegetable, ornamental	Asia
A.ganeticus	Cultivated	Vegetable	Asia
A. viridis	Weed		Africa

Source: Saunders & Becker (1984)

2.1.3 Environment

There are many plants including; sugar cane, sorghum and at least 1000 other plant species that are classified as tropical grasses or arid region plants that can grow in high temperatures with a distinctive leaf anatomy which is referred to as "*Kranz"* anatomy (Brown, 1975; Brown & Hattersley, 1989). This type of anatomy contains two types of chloroplasts within the leaves and causes differences in the plants' photosynthesis. Plants that have this anatomy produce a *4-carbon* compound instead of the normal *3-carbon* compound during the primary stages of the light-independent reactions of photosynthesis. Plants that have this system of carbon production are known as C4-plants and have a higher optimal growing temperature compared to plants that have the *3-carbon* system (Berry 2001; Stern, 2006).

Amaranth is known as a C4-plant, being one of the few dicots which produce a *4-carbon* compound as a first product of photosynthesis (National Research Council, 1984; Grosz-Heilman *et al*., 1990; Stallknecht & Schulz-Schaeffer, 1993; New World Encyclopedia, 2009). The C4 pathway is a modification of the normal photosynthetic process that makes efficient use of the carbon dioxide, available in the air by concentrating it in chloroplasts of specialized cells surrounding the leaf vascular bundles. The photo respiratory loss of carbon dioxide, the basic unit of carbohydrate production is suppressed in C4-plants. Consequently, plants that use C4 pathway can convert a higher ratio of atmospheric carbon to plant sugars per unit of water lost than those possessing the classical C3 (Calvin cycle) pathway (Saunders & Becker, 1984). Through osmotic adjustment, the plants can tolerate some lack of water without wilting or drying. This is an adaptation for surviving periods of drought. The potential ability to photosynthesize at high rates under high temperature is another physiological advantage of C4 photosynthesis (Saunders & Becker, 1984; Stallknecht & Schulz-Schaeffer, 1993; Mshelia & Degri, 2014).

The habitat where amaranth is found differs dramatically and the temperatures that they can grow at ranges from 20-35°C (Grosz-Heilman *et al*., 1990; Guo & Al-Khatib, 2003), however, amaranth grows best when the temperature is at least 21°C. Although *A. hypochondriacu*s and *A. cruentus* tolerate high temperatures, they are not frost hardy and growth ceases practically at about 8°C (Saunders & Becker, 1984; Bermejo & Leon, 1994). Amaranth plants grow very fast, resist drought, heat,

pests and are able to adapt readily to new environments (Grosz-Heilman *et al*., 1990; Graber 2014).

The plants have shown immense adaptation to a wide variety of climates which range from the lowland tropics to cold mountainous conditions (altitudes between 110 and 3000 meters above sea level), from the southwest United States, China, India, Africa, Nepal, South Pacific Islands, Caribbean, Greece, Italy and Russia (Teutonico & Knorr, 1985; Grosz-Heilman *et al*., 1990; Stallknecht & Schulz-Schaeffer, 1993; Cunningham, 2010; Graber, 2014; Mshelia & Degri, 2014). Amaranth adapts so fast because of the amount of seeds produced in nature and because these plants hybridise readily (Cunningham, 2010).

Day length amaranths are sensitive to length of day. For example strains of *Amaranthus hypochondriacus* from the South of Mexico will not set flower in the summer in Pennsylvania (Teutonico & Knorr, 1985). They do, however mature in green houses during the short-day conditions of winter. The reverse happens with *Amaranthus cruentus* from Nigeria. It remains vegetative for a long period in its equatorial home. However, it goes to seed very early when introduced into long day conditions in Pennsylvania and can be used to breed for early maturing traits (Saunders & Becker, 1984).

These plants are also able to grow very well in a variety of soils containing widely varying levels of soil nutrients. Grain amaranth however, requires well drained sites and appears to prefer neutral or basic soils (Saunders & Becker, 1984). For seeds to germinate and establish roots, grain amaranths require well moistened soil, but once seedlings are established, grain amaranths grow well under dry and warm conditions.

2.1.4 Uses of the genus *Amaranthus*

2.1.4.1 As a food crop

Due to the ever increasing human population worldwide, there is a great need for food security, so it is very important to get the maximum yield from food crops (Bhadoria, 2011). The modern world relies on six crops - notably cereals (wheat, rice & maize), root crops, legumes, sugarcane, sugar beets and bananas which provide

the bulk of the worlds' calories and proteins (Grosz-Heilman *et al*., 1990; International Development Research Centre, 2016). In order to increase the food base world-wide, lesser-known or older forgotten crops such as amaranth should be reconsidered (Grosz-Heilman *et al*., 1990). Amaranth is one of the few non-grass 'crops' that has the potential to increase and diversify the food base and also to increase world food production (Grosz-Heilman *et al*., 1990; Yangzhou & Stuttgart, 1999; Graber, 2014).

Grain amaranth belongs in the pigweed family and has the potential to become an alternative crop in different parts of the world (Shroyer *et al*., 1990). Grain amaranth is a non-grass plant and to distinguish it from the grasses, it is called pseudo cereal. Other pseudo cereals include quinoa and buckwheat (Mwangi, 1993). *Amaranthus caudatus*, *Amaranthus cruentus* and *Amaranthus hypochondriacus* have been identified (Tucker, 1986; Yangzhou & Stuttgart, 1999) as having potential to increase world food production. It must be noted that leaves of many cultivated grain species of *Amaranthus* are commonly eaten as a pot herb or leafy vegetable throughout the world (Kauffman & Webber, 1990) thus there is no dividing line between grain and vegetable (leaf) types.

The crude protein content of grain amaranth is shown in table 2.2 and ranges from 13-19 % dry matter, which is among the highest protein levels of grain in the world (Mwangi, 1993). It has a high level of lysine, the amino acid which is usually lacking in the grass family cereals like rice and maize, so amaranth grain plays a role as a nutritional fortifier in cereals for human consumption and animal feeds (Mwangi, 1993). Another limiting amino acid i.e. sulphur amino acid is present in amaranth at 4.4 % (Mwangi, 1993).

The total lipid content of grain amaranths ranges from 5.4-17.0 % dry matter (Saunders & Becker, 1984; Dhellot *et al*., 2006; Gamel *et al*., 2007). Amaranth oil has high levels of unsaturation, about (77 %) similar to wheat (77 %), oat (77 %), corn (78 %), brown rice (75 %) and olive (87 %) (Mwangi, 1993). Amaranth also contains significant high levels of squalene, 6-11.2 % which is considerably higher than usually found in oils from other cereal grains (Saunders & Becker, 1984; Gamel *et al*., 2007). Squalene finds applications in the cosmetic industry and is normally extracted from shark liver (Schwartz, 1988). Uses of amaranth seed oil which

contains mainly non polar lipids especially triglycerides with a high degree of unsaturation have been documented with the seed oil containing three important fatty acids; palmitic (18-22 %), oleic (25-28 %) and linoleic acid (44-50 %) (Tamer *et al*., 2006).

Table 2.2: Composition of species of grain amaranths.

Analyte	***A. cruentus***	***A. edulis***	***A. hypochondriacus***	***A. hybridus***
Moisture (%)	6.23-6.71	9.55-11.6	11.1	9.2
Crude protein (%dmnx6.25)	13.2-17.6	15.8-16.5	13.9-17.3	14.0-18.0
Total lipids (%)	6.3-8.1	6.9-8.1	7.7	-
Crude fibre (%)	3.4-5.3	3.2-5.8	1	6.2-6.4
Crude ash (%)	2.8-3.6	3.2-4.4	3.3-4.1	8.1
Na	31.0	37	6.7-10.0	7.8
K	290	580	-	33.5
Ca	175	36	137-167	17.4
Mg	244	-	292-363	5.96
Fe	17.4	13.1	9.1-21.7	-
Zn	3.7	-	3.6-3.9	-
Cu	1.2	-	0.6-0.8	-
Mn	4.6	-	109-2.9	-
Riboflavin	0.19-0.23	-	0.29	-
Niacin	1.17-1.45	-	1.5	-
Ascorbic acid	4.5	-	2.8	-
Thiamine	0.07	-	0.25	-

Minerals in mg/100g

Source: Becker *et al*, (1981); Dhellot *et al*, (2006).

The high nutritional values of the grains have led to renewed interest in this crop (Aufhammer *et al*., 1998; Yangzhou & Stuttgart, 1999). Ebert *et al*. (2011), confirmed that vegetable amaranth is highly nutritious because of its richness in protein, calcium, iron, vitamin A, C and K, riboflavin (B2), niacin (B3), vitamin B6 and folate (B9).

As a crop plant Amaranth became neglected due to exotic crop plants taking over their original function (Radosavljević, 2006). The market demand for amaranth has fluctuated, however, steady use of this crop for breakfast cereals, snack foods, and more recently, in mass produced multigrain bread products (Myers, 1996) has come to light. A great aspect of amaranth seeds is that these seeds are naturally gluten-free and is considered a healthy alternative to the gluten-containing grains in a gluten-free diet or for people that are gluten sensitive (Alvarez-Jubete *et al*., 2010). When *Amaranthus* flour is mixed with wheat flour, the product has a higher protein value (Yangzhou & Stuttgart, 1999; Radosavljević, 2006).

The promising economic values of the unexplored Amaranth plant in the United States has been recognised by the National Academy of Science. Amaranth is considered an alternative crop today and researchers around the world have focused on improving the agronomic features of the plant which include nutritional quality, and processing of the seeds (Yaacob *et al*., 2004; Radosavljević, 2006). These plants leaves are an inexpensive, rich source of dietary fibre, protein, vitamins as well as a wide range of minerals (Yangzhou & Stuttgart, 1999; Shukla *et al*., 2005; Graber, 2014).

2.1.4.2 Medicinal uses

Amaranthus spp. in particular, *A. cruentus* and *A. hybridus* (Cai *et al.*, 2005; Guerra-Matias & Arêas 2005; Fasuyi 2006, 2007; Odhav *et al.*, 2007) were of great importance in the pre-Colombian American people's diets (Tosi *et al.*, 2001; González *et al*., 2007). The consumption of *A. cruentus* products is advised for patients with celiac disease and, therefore, also for diabetic persons (Guerra-Matias & Arêas 2005). *A. cruentus* contains alkaloids, saponins, tannins and inulin and can be used for many medical uses such as the treatment of tapeworm, and relief of respiratory diseases (Mensah *et al*., 2008).

A. hybridus has been used traditionally for the treatment of liver infections and knee pain and for its laxative, diuretic, and cicatrisation properties (Nacoulma, 1996); the products are used particularly for stomach aches, diarrhoea, and dysentery. *A. hybridus* leaves are used as a vegetable (Dhellot *et al*., 2006) and sauces prepared from this plant are recommended for convalescent patients (Hilou, 2006).

A. caudatus is astringent, anthelmintic and diuretic (Grieve, 1984; Chopra *et al*., 1986). It is used in the treatment of strangury and is applied externally to scrofulous sores (Chopra *et al*., 1986).

The whole plant of *A. hypochondriacus* contains tannins and is astringent. It is used internally in the treatment of diarrhoea and excessive menstruation. It can be used as a gargle to soothe inflammation of the pharynx and to hasten the healing of mouth ulcers, whilst it can also be applied externally to treat vaginal discharges, nosebleeds and wounds (Brown, 1995; Chevallier, 1996).

A liquid extract of *A. blitum* is used for the treatment of mouth, throat and other external ulcers (Grieve, 1984). The plant has a folk reputation for being effective in the treatment of tumours and warts in China (Duke & Ayensu, 2008).

A tea made from the leaves of *A. retroflexus* is used in the treatment of profuse menstruation, intestinal bleeding, diarrhoea and to treat hoarseness (Foster & Duke, 1990; Brown, 1995; Moerman, 1998).

The roots of *A. spinosus* are used in menorrhagia, gonorrhoea, eczema, colic and also used as galactagogue (Banerji, 1978). Leaves and roots are laxative, emollient and used as poultice on abscesses (Baquar, 1989). Leaf extracts had also been used in the treatment of menstrual disorders (Olufemi *et al*., 2003). The plant is extensively used in Chinese medicine to treat diabetes (Lin *et al*., 2005).

Vegetable amaranths also contain phytochemicals that help protect the body from long-term degenerative diseases (Raheena, 2007). The vegetable has been reported to have a high concentration of antioxidant components (Hunter & Fletcher, 2002), and have anti-inflammatory properties (Olumayokun *et al.* 2004).

2.1.5 *Amaranthus cruentus*

A. cruentus also known as the ‘red amaranth’ or the ‘Mexican grain amaranth’ is an annual, pseudo-cereal with broad leaves that is used for its high-protein grain, a leafy vegetable or as a forage crop (Steckel, 2004; Yaacob *et al*., 2004). The grain types have white seeds while the vegetable types (as well as those used to extract red dye) usually are dark seeded. It is probably the most adaptable of all amaranth

species, for example it flowers under a wider range of day lengths than the others (Grosz-Heilman *et al*., 1990).

A. cruentus is an ancient food and found in the famous Tehuacan caves in Central Mexico (Saunders & Becker, 1984). The species is still grown in the region and popped amaranth seed cakes are sold on the streets of the towns (Russell, 2016). *A. cruentus* survived as a green crop in few Indian villages of Southern Mexico and Guatemala and also as a crop used to extract a red dye for colouring corn based foods in the Indian Pueblos of the arid South Western United States, where it probably became established in pre-historic times (Saunders & Becker, 1984). Both the grain and the leaves are used for human and animal consumption (Stallknecht & Schulz-Schaeffer, 1993).

The seeds contain starch in concentrations that range from 48-69%, depending on the species. The flour made from these plants is suitable for biscuits, breads, cakes and many other baked goods. There is no functional gluten in the grains (Teutonico & Knorr, 1985). The protein (12-18%) derives from the amino acid lysine which is normally limiting in most cereal grains, while the grains also contain 5-8% lipids (Stallknecht & Schulz-Schaeffer, 1993; Aufhammer *et al*., 1998; Yangzhou & Stuttgart, 1999; Alvarez-Jubete *et al*., 2010; Graber, 2014).

This plant group is drought tolerant and requires warm conditions (18°C-24°C) to germinate (Grosz-Heilman *et al*., 1990; Yaacob *et al*., 2004). *A. cruentus* is one of the most widely grown species in the world.

2.2 Phytochemistry

Primary metabolism is a complex of metabolic processes used for the performing of essential functioning in the plant; such as photosynthesis, respiration and transport of solutes. These components are universally distributed throughout the plant (Taiz & Zeiger, 2012). Secondary metabolism gives rise to phytochemicals that are not universal, they are also not essential for survival of these plants (Castro *et al*., 2005). It is well-known that plants produce these chemicals to protect themselves against other plants, pests and pathogens and environmental stress (Ferguson *et al*., 2003; Li *et al*., 2010).

Studies have shown that phytochemicals have a beneficial effect on human health and play an active role in amelioration of diseases and insect resistance (Shettel & Balke, 1983; Ferguson *et al.,* 2003). There have been claims by some scientists that many of the diseases affecting humans are the result of a lack of phytonutrients in their diet. Phytonutrients have various health benefits, for example, they may have antimicrobial, anti-inflammatory, cancer preventive, antidiabetic and antihypertensive effects to mention but a few. The phytochemical constituent of a plant will often determine the physiological action on the human body (Pamplona-Roger, 1998). Recently this area of science has received greater attention from researchers and farmers worldwide (de Albuquerque *et al*., 2010).

2.2.1 Chemical composition of amaranth

A literature survey of the genus revealed the presence of carotenoids, steroids (Bishop & Yokota, 2001; Oboh *et al*., 2008; Maiyo *et al*., 2010), terpenoids (Connick *et al*., 1989), ascorbic acid, betacyanins (Cai *et al*., 1998), α-spinasterol, spinoside, amaranthoside, amaracine (Shah, 2005) and phenolic compounds (Kraujalis *et al*. 2013). The methanol extracts of, *Amaranthus caudatus* also contained tannins and phlobatannins, saponins (Maiyo *et al*., 2010) and triterpene saponins (Mroczek, 2015) were detected in *Amaranthus spinosus*. The bioactivity of saponin mixtures or individual saponins includes cytotoxic, immunomodulatory, hepatoprotective, antidiabetic, hypolipidemic, antiosteoporosis, antiviral, antifungal and anthelmintic actions. Herbal and edible Amaranthaceae plants can be considered as promising and highly available sources of biologically active triterpene saponins.

2.2.1.1 Carotenoids

Amaranth vegetable have been documented to contain a higher carotenoid content than most vegetables (Su *et al*., 2002). Carotenoids are yellow, red and orange pigments present in many fruits and vegetables. In dark green leafy vegetables, carotenoids are masked with the presence of chlorophyll. Leafy vegetables are a rich source of many carotenoids (Kimura & Rodriguez-Amaya, 2003; De Oliveira & Rodriguez-Amaya, 2007). More than 700 carotenoids have been identified in nature, with β-carotene being the most familiar carotenoid. The most commonly studied include lutein, zeaxanthin, lycopene, β-carotene, α-carotene, and β-cryptoxanthin

(McLauren & Frigg, 2002). Besides the well-known provitamin A activity of some carotenoids (Tanumihardjo, 2008), they act as powerful antioxidants and are believed to protect the body against free radical attack and hence reduce the incidence of cataracts, heart disease and other degenerative diseases (Krinsky, 1993). Carotenoids including α -carotene, β-carotene and β-cryptoxanthin can be converted into Vitamin A, while lycopene, lutein, and zeaxanthin have no vitamin A activity.

It is not clear which specific carotenoids are most important in reducing the cancer risk. Previously, scientists believed that β-carotene had important cancer prevention properties (Stähelin *et al*., 1991; van Poppel & Goldbohm, 1995). In several recent studies, however, β-carotene supplements did not lower the risk of cancer (Goralczyk, 2009). The antioxidant activity of carotenoids differs among the different compounds. Studies have shown that the singlet oxygen quenching capacity of lycopene is twice that of β-carotene and ten times that of tocopherol (Di Mascio *et al*., 1989). Several studies on the bioavailability of β-carotene from vegetables in the human diet have shown that in broccoli it ranges from 22-24%, in carrots 19-34%, and in leafy vegetables it ranges from 3-6%. Studies have also shown that combination of fatty foods with carotenoid rich vegetables enhanced carotenoids uptake (Brown *et al*., 2004; Ribaya-Mercado *et al*., 2007). Most recent studies have shown that the bio-availability of lycopene from tomato has increased dramatically by heat treatment in the presence of oil. For example, lycopene was found to be more bio-available from tomato paste than from fresh tomato due to heat treatment and presence of oil content in the paste (Unlu *et al.*, 2007). Carotenoids are powerful antioxidants that may reduce the incidence of age-related diseases such as cancer and coronary heart disease.

Food carotenoids are usually C40 tetraterpenoids built from eight C5 isoprenoid units, joined so that the sequence is reversed at the centre. Although commonly thought of as plant pigments, carotenoids are also encountered in some animal foods. Animals are incapable of carotenoid biosynthesis, thus their carotenoids are diet derived, selectively or unselectively absorbed, and accumulated unchanged or modified slightly into typical animal carotenoids. Leaves have a strikingly constant

carotenoid pattern, the main carotenoids being lutein (about 45%), beta-carotene (usually 25–30%), violaxanthin (15%), and neoxanthin (15%) (Britton, 1991).

The carotenoid composition of foods are affected by factors such as cultivar or variety; part of the plant consumed; stage of maturity; climate or geographic site of production; harvesting and postharvest handling; processing and storage (Gross, 1987, 1999; Rodriguez-Amaya, 1993). Carotenoids are insoluble in water and soluble in organic solvents, such as acetone, alcohol, ethyl ether, chloroform, and ethyl acetate. They are readily soluble in petroleum ether, hexane, and toluene; xanthophylls dissolve better in methanol and ethanol. Crystalline carotenoids may be difficult to dissolve in the above solvents but do dissolve in benzene and dichloromethane (Schiedt & Liaaen-Jensen, 1995).

Solubility of both β-carotene and the xanthophyll lutein in tetrahydrofuran has been shown to be excellent (Craft & Soares, 1992). Carotenoids in solution obey the Beer-Lambert law—their absorbance is directly proportional to the concentration. Thus, carotenoids are quantified spectrophotometrically. The highly unsaturated carotenoid is prone to isomerization and oxidation. Heat, light, acids, and adsorption on an active surface promote isomerization of *trans* carotenoids, their usual configuration, to the *cis* forms. This results in some loss of colour and provitamin A activity. Oxidative degradation, the principal cause of extensive losses of carotenoids, depends on the availability of oxygen and is stimulated by light, enzymes, metals, and co-oxidation with lipid hydroperoxides. Formation of epoxides and apocarotenoids (carotenoids with shortened carbon skeleton) appears to be the initial step. Subsequent fragmentations yield a series of low-molecular-weight compounds similar to those produced in fatty acid oxidation. Thus, total loss of colour and biologic activities are the final consequences (Rodriguez-Amaya, 1999).

2.2.1.2 Phenolic compounds

These compounds are a class of the most important and most common plant allelochemicals in an ecosystem and are found throughout the plant kingdom (Bhattacharya *et al*., 2010). The compounds contain a hydroxyl group (-OH) bonded directly to an aromatic hydrocarbon group (Balasundram *et al*., 2006; Li *et al*., 2010). This is a diverse group of compounds, it is broadly divided into non-soluble

compounds such as condensed tannins, lignins, and cell-wall bound hydroxycinammic acids and the soluble phenolics such as phenolic acids, phenylpropanoids, flavonoids and quinones (Rispail *et al*., 2005). Phenolic compounds are secondary metabolites that are the derivatives of the pentose phosphate, shikimate and phenylpropanoid pathways (Balasundram *et al*., 2006).

In plants there are many types of phenolics, all with different functions in the plant, including pigmentation and defence e.g.

- **Simple phenylpropanoids:** Compounds such as caffeic acid and ferulic acid occur in soil in appreciable amounts and have been shown to inhibit the germination and growth of many plants (Inderjit *et al.*, 1995).
- **Flavanoids:** Flavonoids are a ubiquitous group of polyphenolic substances which are present in most plants. There are four major groups of flavonoids: 1) anthocyanins are coloured flavonoids that attract pollinators; 2) flavones and 3) flavonols may protect against ultraviolet light (Li *et al.*, 1993); 4) isoflavonoids are mostly found in legumes and have several different biological activities. Some have been shown to have anti-inflammatory, anti-allergic, anti-neoplastic, antiviral, anti-thrombotic and vasodilatory activities (Miller, 1996). Good correlation between the total flavonoids content and antioxidant activity has been shown, (Ayoola *et al*, 2008), indicating that the flavonoids contribute in free radical scavenging. In the past few years, isoflavonoids have become best known for their role as phytoalexins, antimicrobial compounds synthesized in response to bacterial or fungal infection that help to limit the spread of the invading pathogen (Cheng *et al*., 2007; Bhattacharya *et al*., 2010).
- **Tannins:** Tannins have a strong deleterious effect on phytophagous insects and affect the insect growth and development by binding to proteins, reduce nutrient absorption efficiency, and cause midgut lesions (Sharma & Agarwal, 2011). Tannins are astringent (mouth puckering) bitter polyphenols and act as feeding deterrents to many insect pests. They precipitate proteins non-specifically (including the digestive enzymes of herbivores), by hydrogen bonding or covalent bonding of protein-NH_2 groups. In addition, tannins also chelate the metal ions, thereby reducing their bioavailability to herbivores.

When ingested, tannins reduce the digestibility of the proteins thereby decrease the nutritive value of plants and plant parts to herbivores. Role of tannins in plant defence against various stresses and their induction in response to insect damage has been studied in many plants (Barbehenn & Constabel, 2011).

2.2.1.3 Steroids

The most prominent plant steroid is brassinolide ($C_{28}H_{48}O_6$), part of a larger class of plant steroids called brassinosteroids. This steroid is important to the development of plant cells and promoting the plant's growth (Bishop & Yokota, 2001; Bishop & Koncz, 2002).

2.2.1.4 Terpenoids

Among plants secondary metabolites terpenoids are the most structurally diverse group. Plants use some terpenoid metabolites for many different basic functions in the growth and development but use the majority of these metabolites for more specialized chemical interactions and for protection in both abiotic and biotic environments (Tholl, 2015). Plants accumulate terpenes for herbivore defence as well as emit volatile blends in response to the herbivory and many other stresses. Due to the complexity of these volatile blends, it is hard to assign a specific function to a certain terpene. Monoterpenes and diterpenes, main compounds of essential oils, act as allelopathic agents, attractants in plant-plant or plant-pathogen/herbivore interactions as well as repellents (Graβmann, 2005).

2.2.1.5 Ascorbic acid

Ascorbic acid functions as a redox buffer and as a cofactor for the enzymes involved in regulating photosynthesis, hormone biosynthesis, and regenerating other antioxidants (Gallie, 2013). This chemical is proposed to function in photosynthesis as an enzyme cofactor (including the synthesis of ethylene, gibberellins and anthocyanins) and in the control of cell growth. In photosynthesis this chemical acts in the Mehler peroxidase reaction with ascorbate peroxidase to regulate the redox state of the photosynthetic electron carriers and as a cofactor for the violaxanthin de-epoxidase, the enzyme involved in xanthophyll cycle-mediated photo-protection

(Smirnoff & Wheeler, 2000). In cell growth ascorbate is a cofactor that plays a role in make-up of cell walls, which is important for cell division and expansion, with high concentrations of ascorbate oxidase activity in the cell wall there is a positive correlation with rapid cell expansion (Zhang, 2013).

2.2.1.6 Betacyanins

Betalains are alkaloid pigments; they appear red to red-violet in colour. Betacyanins play a role in the photo-protective function within plants. *Amaranthus* pigments are red-violet betacyanins, which helps with photoprotection (Cai *et al*., 1998; Nakashima *et al*., 2011). Betacyanin acts as a filter for light intensity that moves into the leaf tissue to reduce reactive oxygen species (ROS) generation, this also acts as a ROS scavenger under stress conditions (Nakashima *et al*., 2011). Amaranthine, is a betacyanin which is a plant antioxidant and pigment found in amaranth (Shah, 2005).

2.2.1.7 Alpha spinasterol

This chemical is a phytosterol that is found in a variety of plants sources such as spinach, cucumber, alfalfa meal, pumpkin seeds and senega root (Biopurify Phytochemicals Ltd., 2015). Phytosterols, consist of plant sterols and stanols which are steroid compounds which are similar to cholesterol which occurs in plants.

2.2.1.8 Spinoside, Amaranthoside and Amaracine

These compounds were isolated from *Amaranthus spinosus* by Shah, 2005. Spinoside was identified as a derivative of apeginin through gas chromatography (Shah, 2005).

2.2.1.9 Antioxidant nutrients

Various abiotic stresses lead to the overproduction of reactive oxygen species (ROS) in plants which are highly reactive and toxic and cause damage to proteins, lipids, carbohydrates and DNA which ultimately results in oxidative stress (Bhattachrjee, 2005). The antioxidant defence machinery protects plants against oxidative stress damages and are mainly due to the presence of carotenoids, ascorbic acid, glutathione, alkaloids, non-protein amino acids, α-tocopherols and phenolic

compounds such as flavonoids, phenolic acids, tannins and phenolic diterpenes (Polterait, 1997; Mittler *at al*., 2004).

Vegetable amaranth has been shown to have good antioxidant activity, which in turn prevents the body from harmful radicals hence prevent some chronic diseases. The antioxidant potential of the extracts would be responsible for the prevention of the cardiovascular and neurodegenerative diseases (Heim *et al*., 2002), bones diseases (Govindarajan *et al.*, 2005) and cancer (Kawanishi *et al.*, 2001). Aqueous extracts of *A. hybridus* have been shown to have anti-anemic activity on rabbits (Ogbe *et al.,* 2010). Studies carried out by Ozsoy *et al*. (2009) showed that stems with leaves and flowers of species of the same family as *A. lividus* seemed to be good sources of natural antioxidants. Oloyede *et al*., (2013) reported the highest concentration of antioxidants at maturity stage in *A. cruentus.*

2.3 Allelopathy

The chemicals that are produced for the allelopathic function of the plant are called secondary metabolites, which are investigated by phytochemists (Li *et al*., 2010). Allelochemicals in majority are secondary metabolites released into the environment especially the rhizosphere. Allelochemicals are a large, diverse array of organic compounds that have no direct function in growth and development. Many different secondary metabolites-e.g., phenolics, terpenoids, alkaloids, polyacetylenes, fatty acids, and steroids-can act as allelochemicals (Rice, 1984; Waller, 1987; Inderjit *et al*., 1995). These chemicals are present in various plant parts; however, their mere presence does not establish allelopathy (Putnam & Tang, 1986; Heisey, 1990). To demonstrate their involvement in allelopathy, it is important to establish 1) their direct release or indirect origin from plant-derived materials in the environment and 2) that the chemicals are present in sufficient quantities and persist for a sufficient time in the soil to affect plant species or microbes (Putnam & Tang, 1986).

In 1996, the International Society of Allelopathy defined allelopathy' as any process involving secondary metabolites produced by plants, microorganisms, viruses or fungi that influence the growth and development of biological and agricultural systems' (Rice, 1984; Ferguson *et al.,* 2003; Allelopathic Journal, 2006; Ferreira & Reinhardt, 2010; Li *et al*., 2010). Allelopathy also refers to the beneficial or harmful

effects of one plant on another; this can be from both crops and weeds (Ferguson *et al.,* 2003).

According to Amini & Ghanepour (2013), allelopathy, is the chemical inhibition of one plant species by another, this represents a form of chemical warfare among plants competing for limited light, water and nutrient resources.

Allelopathy is the process through which certain plants release chemicals (phytotoxins) that will affect other species of plants growing in the same location. These chemicals are usually detrimental to the affected plant (Pratley 1996; Colquhoum 2006; D'Abrosca *et al*., 2006; Baghestani *et al*., 1999; Faravanim *et al*., 2008; Ferguson *et al.,* 2003). Li *et al*., (2010), also referred to allelopathy as a "phenomenon involving either direct or indirect and either beneficial or adverse effects of a plant (including microorganisms) on another plant through the release of chemicals in the environment". Allelopathy has been noted in literature for more than 2,000 years in respect to plant interference (Li *et al*., 2010). This process usually gives the allelopathic plant a competitive advantage (Li *et al*., 2010). As this phenomenon can have positive or negative effects, there has been much debate on the matter of using allelochemicals for crop practices, however, and the strong scientific evidence in the last few years has raised questions regarding the credibility in this area of study (Pratley, 2006; Shahrokhi *et al*., 2011).

Allelochemical compounds and their modes of action are very diverse, and these chemical compounds have potential for development of future 'natural' herbicides.

The first time allelopathy was described was by the Romans as a process resulting in the "sickening" of the soil; this was seen in chickpeas (*Cicerarictinum* L.), (Weston 2005). The term *allelopathy* is from the Greek-derived compounds *allelo* and *pathy* (meaning "mutual harm" or "suffering") and was first used in 1937 by Austrian scientist Hans Molisch in his book *Der Ein flusseiner Pflanze auf die andere – Allelopathie* (The Effect of Plants on Each Other), (Ferguson *et al.,* 2003; Sotomayor & Lortie, 2014; Bhadoria, 2011). The plant-plant interference has been known for some time, but only in 1937 did the Austrian plant physiologist, Hans Molisch, gave it the formal name "allelopathy" and because of this he has become known as the father of allelopathy (Li *et al*., 2010).

Theophrastus, "the father of botany", was the person who recorded the earliest observation of the weed and crop allelopathy, he wrote in his botanical works about how chickpea "exhausted" the soil and destroyed weeds in 300 B.C. (Li *et al*., 2010). The famous Roman politician and writer, Cato the Elder (234-140 B.C.) who farmed when he was younger, wrote in his book about how chickpea and barley "scorch up" the corn land. In this book he also mentioned that walnut trees were also toxic to other plants (Li *et al*., 2010).

The phenomenon occurs because inhibitory substances are released directly from the living plant into the surrounding environment through root exudation, leaching and volatilization, and when the plant dies and decomposes (Rice, 1984; Minorsky, 2002; Bertin *et al*., 2003; Weston, 2005). The most frequently reported morphological effect from allelochemicals on susceptible plants are inhibited or retarded seed germination and there are effects on coleoptile elongation as well as shoot and root development (Ghafarbi *et al*., 2012).

Allelopathy has been said to be involved in many natural and manipulated ecosystems and plays a role in the change (evolution) of different plant communities (Ding *et al*., 2007).

Allelopathy is found in many plant species (Hasanuzzaman, 2014). Allemann & Denner (2006) reported that allelopathic effects have been noted in many species including crop plants, perennial weeds and annual weeds. Allelochemicals or allelopathic chemicals are secreted by these plants that can inhibit growth and yield of the other plants growing in the surrounding area or plants that are grown in the same area after the allelopathic species. Allelochemicals can be released from plant residues by decomposition or leaching by water (Allemann & Denner, 2006; Hasanuzzaman, 2014). Allelochemicals are found in all parts of the plant. These compounds can also be released by volatilization, root exudates from both the live and decaying plants as seen in figure 2.1 (Hasanuzzaman, 2014).

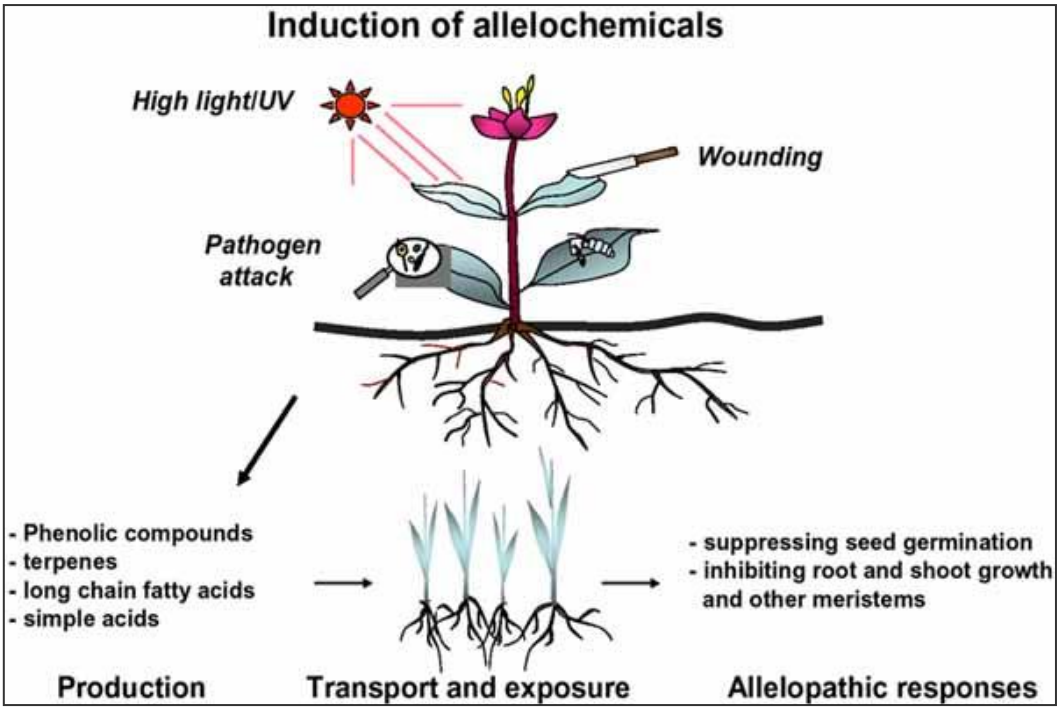


Figure 2.3: The production of allelochemicals through different stresses (Kim & Shin, 2003).

Allelopathic compounds prevent the germination and development of certain plants and this can lead to a decrease in overall yield, quality and also harvest efficiency of agricultural crops (Putnam, 1988; Guo & Al-Khatib, 2003; Ferguson *et al.,* 2003). The known sites of action for some of these allelochemicals include cell division, pollen germination, nutrient uptake, photosynthesis and some enzyme functions (Ferguson *et al.,* 2003). However, allelopathy can play a positive role in the management of natural and agricultural systems (D'Abrosca *et al*., 2006). Interest in these allelochemicals have become important because of the possible application of allelopathy as an alternative to synthetic herbicides for the control of weeds (Wu *et al*., 2001; Shettel & Balke, 1983), allelopathic compounds are often considered to be plant-produced herbicides so these compounds are natural (Colquhoum, 2006). Natural products has received more interest in recent years as sources of molecular bioactive skeleta for use as natural herbicides (D'Abrosca *et al*., 2006) and pesticides (Ghafarbi *et al*., 2012).

There are about 250 species of allelopathic weeds that cause problems in the agricultural crops production and in turn will affect food prices because of yield loss. The main effect of weeds is their ability to seriously compete with crop species

because they are very adaptive to the surrounding environment; therefore they are known as an important crop yield reducing factors (Shahrokhi *et al*., 2011). The weeds will not only compete with the crop plants for food, water, sunlight and space, they will also reduce the crops' growth and yield by releasing their allelopathic substances (Khanh *et al*., 2006).

The effects of allelopathic compounds can be seen in the loss of seed germination, seedling growth, leaf area, dry matter production and the amount of pigments produced, carbohydrates and proteins, and in the end can halt the growth and development of other plants (Rahimzadeh *et al*., 2012). Due to the increase in the human population it has become important to reduce environmental effects and cost of crop protection is a major concern (Baghestani *et al*., 1999). Weeds can have a high toll on crop plant yields, leading to losses of billions of dollars around the world (Letournea *et al*., 1956; Shettel & Balke, 1983). Due to the effect that weeds have upon crops plants is the reason why it has become an issue to find alternative ways of weed management (Weston, 2005; Ferreira & Reinhardt, 2010).

Allelopathic inhibition is very complex and it involves the interaction of different classes of chemicals which include: phenolics, flavonoids, terpenoids, alkaloids, steroids, carbohydrates and amino acids and sometimes when different chemicals mix there may be a greater allelopathic effect than if there is a single compound alone (Heisey, 1997; Ferguson *et al.,* 2003).

Physiological and environmental stresses e.g. pests and diseases, solar radiation, herbicides, minimum nutrients, moisture and temperature, will have an effect on the allelopathy of weeds (Ferguson *et al.,* 2003).

According to other scientists allelochemicals are responsible for inhibition of plants. It has also been seen that natural products that are derived from plants have become more important in the modern world, and this is because these products have shown diverse pharmacological properties which include antioxidant and antitumor activities (Claro de Souza *et al*., 2011; Mlakar *et al*., 2012; Rahimzadeh *et al*., 2012).

2.3.1 Amaranth allelopathy

Many studies on the allelopatic effect of weed amaranth plants which include redroot pigweed (*A. retroflexus*) are available, but there is a lack of information on this topic for cultivated (grain) amaranth (Fomsgaard *et al*., 2010; Mlakar *et al.,* 2012).

When dried stem tissues of Palmer amaranth was added into soil within Petri dishes, severe inhibition in the growth of roots and stems of grain sorghum were noticed (Menges, 1988). The study also displayed root growth inhibition in the Grand Slam cultivar of cabbage.

Extracts of the shoots of redroot pigweed (*A. retroflexus*) reduced the germination of cabbage and eggplant seeds when studies were done in Petri-dishes (Qasem, 1995). *A. retroflexus* residues that were mixed with soil showed severe reduction in germination of cucumbers, squash cabbage, carrot, cauliflower and eggplant seeds as well the delayed germination in both peppers and tomato seeds (Qasem, 1995). Qasem (1995), also reported that squash seedlings that were grown in pots after *A. retroflexus,* showed lower dry weights, leaf surface area and the seedlings showed signs of nutrient deficiencies. Those results proved the allelopathic influence of *A. retroflexus* on several vegetable crops and the difference in sensitivity of the crop species under laboratory conditions. In all experiments by Qasem (1995), roots were more sensitive than shoots to allelopathic effects and both positive and negative effects were observed. Furthermore, Shahrokhi *et al*., (2011) indicated different levels of susceptibility of germination and growth between five barley cultivars exposed to aqueous extracts of the leaves, stems and roots of *A. retroflexus.*

Three amaranth species (pigweeds) namely *A*. *retroflexus*, *A*. *blitoides* and *A*. *gracilis* were investigated by Qasem (1995) for their allelopathic effects on wheat under laboratory, glasshouse and field conditions. Tests done under laboratory conditions showed that extracts from fresh shoots and roots of all the species reduced the germination, coleoptile and root lengths, as well as the dry weight of the wheat seedlings. The severity of inhibition was dependent on concentration, whereas wheat shoot growth was promoted when exposed to the lower concentrations of the amaranth shoot extract. They also found that fresh plant extracts were more phytotoxic than the dried plant extracts and that shoot extracts had more severe

effects than the root extracts. In the pot trials Qasem (1995), noted that the dried shoot extract of *A. gracilis* promoted the shoot and root growth of the wheat seedlings, while *A*. *retroflexus* and *A*. *blitoides* at 8 g kg^{-1} dried material both reduced the germination and growth of the tested wheat seedlings. The roots of the wheat seedlings appeared to be more sensitive to the allelopathic effect of these weeds. When these weeds were investigated under field conditions, it was found that *A. retroflexus and A. blitoides* reduced the height, grain and straw yield of the wheat whereas *A*. *gracilis* residues stimulated the plant height and increased overall yield of wheat (Qasem, 1995).

Obaid & Qasem (2005), studied the allelopathic effects of *A*. *gracilis* on cabbage, carrot, cucumber, onion, pepper, squash and tomato under glasshouse conditions. It was found that the root exudates that were released into the soil had varying effects on the different vegetable crops. The roots of the tested vegetables were more sensitive than the shoots, however both positive and negative results were found depending on the concentration and growth medium used.

Root exudates of *A. retroflexus* were used against the common bean (Amini *et al*., 2009) in an equal-compartment-agar-method (ECAM) under laboratory conditions (Amini *et al*., 2012). Results showed that the amaranth had an inhibitory effect on shoot and root length and dry mass of the beans. In a pot experiment by Amini *et al*. (2013), the influence of extracts of different *A. hybridus* plant parts on red, white and pinto beans were examined. Extracts made from amaranth shoots suppressed the leaf number and stem elongation of the beans. The shoot extract also impaired both vegetative and reproductive growth in the beans (Amini *et al*., 2013).

Allelopathy of different amaranth species were verified against maize. Konstantinović *et al*. (2014) tested maize (*Zea mays*) against the allelopathic effects of aqueous extracts from leaves, stems and roots of *A. retroflexus*. Leaf and stem extracts of 100 g L^{-1} inhibited the epicotyls, while the hypocotyls were not affected by this concentration. Lower concentrations (25 g L^{-1} and 50 g L^{-1}) however, inhibited hypocotyl growth of the maize significantly. Root extracts showed inhibitory effects on the growth of the maize epicotyls at 25, 50, 75 and 100 g L^{-1} whereas it had no effect on the hypocotyl length. In a study by Samad *et al*. (2008), the influence of aqueous extracts and dry material of *A. spinosus* on maize were done and was

found that both inhibited seed germination by 73%. *Amaranthus tricolor* also inhibited seed germination and seedling growth of maize when using aqueous extracts from the root, stems and leaves (Dhole *et al*., 2013).

In a pot study done by Sadaqa *et al*. (2010), the incorporation of residues of *A. graecizans* at 100 g kg^{-1} of soil, inhibited the germination and growth of onion. The root exudates of *A. graecizans* were most inhibitory to onion seed germination, while foliage leachates reduced the onion height and root length by 59% and 64% of the control, respectively.

Aqueous extracts from fresh roots, stems, leaves, and inflorescence with seeds of the weedy *A. retroflexus* and the grain amaranth *A. cruentus*, suppressed germination of garden cress (Mlakar *et al*., 2012). However, the leaf extracts of both species and the inflorescence extracts of grain amaranth, proved to be more effective. Mlakar *et al*., (2012) concluded that the effects of grain amaranth on germination and early growth of garden cress were concentration dependent, and rest strongly on the plant parts assayed and that experiments under field conditions is necessary.

Radicle length of the maize is affected by *Amaranthus spinosus.* On the other hand, germination, seedling growth and dry matter production were affected maximum by dried mass of stems of weed species. The results demonstrated the allelopathic potential of *Amaranthus tricolor* and suggested that those weed species may affect maize seedling growth and development due to the inhibitory effect of allelochemicals, which are present in the dried parts and aqueous extracts of the weed (Dhole *et al*., 2013).

Sultana *et al*. (2012) found that water extracts of the shoots and leaves of *A. viridis* reduced germination and organ length of canola and ryegrass when experiments were carried out in Petri-dishes (Sultana *et al*., 2012). *A. viridis* root and shoots also exhibited inhibitory effects on the germination of sorghum as well as inhibition of growth in maize (Dhole *et al*., 2013).

Allemann & Denner (2006), carried out an allelopathic study on the influence of *A. cruentus* residues in soils and what the consequences were on the succeeding plant growth of two tomato (*Lycopersicon esculentum* Mill.) cultivars. The results indicated

that plants of both cultivars showed inhibition of growth, including plant height, number of leaves, leaf area and fresh plant mass. Researchers suggested that *A. cruentus* plants or residues secrete a compound(s) that is allelopathic to tomato plants (Allemann & Denner, 2006).

2.4 Effect on agriculture

The Amaranth family contains at least 20 weedy members that cause issues in agronomic crop production of vegetable (Webster, 2006) and row crops (Sellers *et al*., 2003) throughout the United States and much of the world. Redroot or common pigweed (*A. retroflexus*), smooth pigweed (*A. hybridus*), Powell amaranth (*A. powellii*), Palmer amaranth (*A. palmeri*), common waterhemp (*A. rudis*), and tall waterhemp (*A. tuberculatus*) are primarily weedy pests that grow in between cultivated crops (Steckel 2004; Guo & Al-Khatib, 2003). Palmer amaranth (*A. palmeri*) and waterhemp (*A. tuberculatus*) are dioecious members of the Amaranth family, and redroot pigweed (*A. retroflexus*) is a monoecious member (Steckel, 2007).

Palmer is native to the southwestern United States and northwestern Mexico, and waterhemp is native to the mid-western United States. Redroot pigweed is native to the central and eastern United States (Sauer, 1957). Yield losses in soybeans from Palmer amaranth, waterhemp, and redroot pigweed can be up to 78%, 56%, and 38%, respectively (Bensch *et al*., 2003).

There are several factors that contribute to the success of these weeds. A single large plant can mature 100,000–600,000 seeds, and populations of 0.1–1 plants per square foot can shed 10,000–45,000 seeds per square foot, or 0.4–2 billion per acre (Massinga *et al*., 2001; Sellers *et al*., 2003). This prolific seed production makes pigweeds especially difficult to manage, since successful maturation of just one plant per 10,000 emerging seedlings can allow pigweed populations to increase several fold from one year to the next.

Seeds also have a long germination window. Keeley *et al*. (1987) observed emergence of Palmer amaranth from March until October. A rapid growth rate also aids in *Amaranthus* species' ability to compete well with soybeans. Palmer amaranth performs C_4 photosynthesis at a very high rate relative to other C_4 species

(Ehleringer, 1983). Pigweed problems have increased in no-till production systems with conventional herbicides, which leave weed seeds at the surface and select for herbicide-resistant populations (Sellers *et al*., 2003). However, high pigweed populations can occur on organic and non-organic farms, and in conventional, conservation, and no-till systems. The presence of herbicide resistance in populations also gives pigweed species a competitive advantage. This put strain on the agricultural sector due to yield reduction of crop plants which can lead to financial losses (Letournea *et al*., 1956; Putnam 1988, Stallknecht & Schulz-Schaeffer 1993; Graber 2014).

On the other hand, amaranth can be used to control other weed species. In a study done by Fomsgaard *et al*. (2010), it was found that the presence of amaranth seeds did not affect weed seed germination; however, root and especially shoot lengths of the small-seeded species *Veronica agrestis* and *Poa annua* were reduced by up to 50%. Due to amaranths ability to control other weed species, extracts as well as plant material could be used as a possible natural herbicide in the war against other weeds.

2.4.1 Crop rotation

Crop rotation is the process by which farmers rotate different types of crops in different seasons. This process is used in order to decrease the chances of the same crop to stop degradation of the soil. Nevens & Reheul (2001) found in long term studies that crop rotation with or without legumes were essential to maintain high production levels of maize. Crop rotation affects the yields of cultivated plants.

Given amaranth's advantages as a crop, its unique nutritional properties and use as food and feed (Ayo, 2001; Bavec and Bavec, 2006; Grobelnik *et al*., 2009), grain amaranth is receiving increasing attention as an alternative crop worldwide. In a study done by Myers (1998) to evaluate amaranth as alternative crops in various rotation combinations with maize (*Zea mays*), soybeans (*Glycine max*), and wheat (*Triticum aestivum*), it was found that amaranth had no noticeable allelopathic effects on the succeeding crop, and amaranth crop residue did not present a physical problem for planting and stand establishment of the next crop. However, later studies

done by Mlakar *et al.* (2012) showed that cultivated amaranths possess allelopathic properties which can be problematic in crop rotation practices.

2.5 Environmental factors

It is known that plants have the ability to defend themselves against influences (stresses) that could be harmful to them. Stress is usually defined as an external factor that exerts a disadvantageous influence on the plant. There are two main environmental factors that control the environment in which plants grow, namely: abiotic and biotic factors. Many people believe that environmental stress usually, but not always, increases allelopathic properties of the targeted plants and this is done by the increased concentrations of allelochemicals and/or the lowering of the targeted plants resistance to the allelopathic plant (Gawronska & Golisz, 2006).

2.5.1 Abiotic factors

These factors are non-living; this includes rain, soil, fire, drought, salinity, extreme temperature, chemical toxicity and oxidative stress. All these stresses can be a serious threat to agriculture and because of these the land can also be degraded (Wang *et al*., 2003). These types of stresses is the primary cause of crop loss throughout the world, according to Wang *et al* (2003), this can reduce the average yields for most major crops by more than 50%.

Abiotic stresses can lead to morphological, physiological; biochemical and molecular changes within plants and these changes can have negative effects on plant growth and production (Wang *et al*., 2003).

Various authors reported on abiotic factors influencing the content of secondary metabolites in plants (Gobbo-Neto & Lopes, 2007; Ramakrishna & Ravishankar, 2011; Gouvea *et al*., 2012). UV-light, nutrient deficiencies, temperature as well as herbicide treatment increased the accumulation of phenylpropanoids (Ramakrishna & Ravishankar, 2011). These authors also referred to the accumulation of amides and polyamines in stressed bean and tobacco, indicating the anti-oxidative role of these secondary metabolites. Rivero *et al*. (2001) found that temperature stress induces the production of phenolic compounds including flavonoids and

phenylpropanoids. Heat and cold stress increased PAL activity resulting in an increase in the production of phenolic compounds (Rivero *et al*., 2001).

2.5.1.1 Temperature

Most tissues of higher plants are unable to survive extended exposure to temperatures above 45°C. Non growing cells or dehydrated tissues (e.g., seeds and pollen) can survive much higher temperatures than hydrated, vegetative, growing cells (Levitt, 1980) C3- and C4-plants rely on transpirational cooling to lower leaf temperature. Both photosynthesis and respiration are inhibited at high temperatures (Björkman *et al*., 1980). The stability of various cellular membranes is important during high-temperature stress. Fluctuating fluidity of membrane lipids at high temperatures just as it is during chilling is correlated with loss of physiological functions (Raison *et al*., 1982).

In order to sustain temperature stress, plants use different protective mechanisms ranging from structural to biochemical. One of the biochemical mechanisms could be through synthesis of heat shock proteins (Wang *et al*., 2004). Other important biochemical defence mechanism is through enhanced production of secondary compounds (Vickers *et al.,* 2009; Fares *et al*., 2010). Volatile organic compounds (e.g., isoprene) are suggested to maintain membrane integrity by linking lipid-lipid, lipid-protein, and/or protein-protein attachments so that the chloroplast membrane remains intact (Renneberg *et al*., 2006). Isoprenoids and phenolics may also alleviate cellular oxidative stress, and help to avoid membrane damage (Appel, 1993; Loreto *et al*., 2004). Hence, they enable plants to be thermos-tolerant and maintain their photosynthesis at elevated temperatures (Loreto *et al*., 1998; Peñuelas & Llusià, 2002). In cold temperatures the metabolic rate and growth of plants will start to slow down. When plants are exposed to very cold temperatures fruit ripening and seed germination is most severely inhibited (Bhattacharya *et al*., 2010).

2.5.1.2 Water

Water is also an important environmental factor for plants concerning germination and growth. Seeds need to be fully imbibed with water before they are able to germinate. When there is water stress, such as drought, plants adapt using

extremely complicated changes in physiological defences. Defences include inhibition of shoot growth and simultaneously stimulates the growth of roots, this is due to the fact that the roots are ‘searching; for water in deeper soil. A decrease in shoot length results in lowering transpiration rate to save water within the plant (Stern, 2006).

Less than 1% of the water a plant absorbs is used for photosynthesis. The other water is used in transpiration and other physiological reactions. If there is a drought or a low water table, photosynthesis will indirectly be affected, the stomata will close and because of this there will be a reduced supply of carbon dioxide resulting in a lower photosynthesis rate (Stern, 2010).

Drought stress markedly enhanced the total concentrations of monoterpenes and resin acids in the main stem wood of Scots Pine and Norway Spruce Seedlings (Turtola *et al*., 2003). Results of investigations conducted on the effect of water deficit imposed to potted *Prunella vulgaris* L. a Chinese plant of medicinal and industrial importance, demonstrated increased levels of the phenolic triterpenes rosmarinic acid, ursolic acid and oleanolic acid (Chen *et al*., 2011). Drought also caused increased accumulation of phenolic compounds (ferulic acid) in the leaves of triticale seedlings (Hura *et al*., 2009) and enhancement of total phenolic contents of *Trachyspermum ammi* leaves (Azhar *et al*., 2011). The main physiological and biochemical known mechanisms triggered by water stressed plants are illustrated in Figure 2.2 (Zingaretti *et al*., 2013).

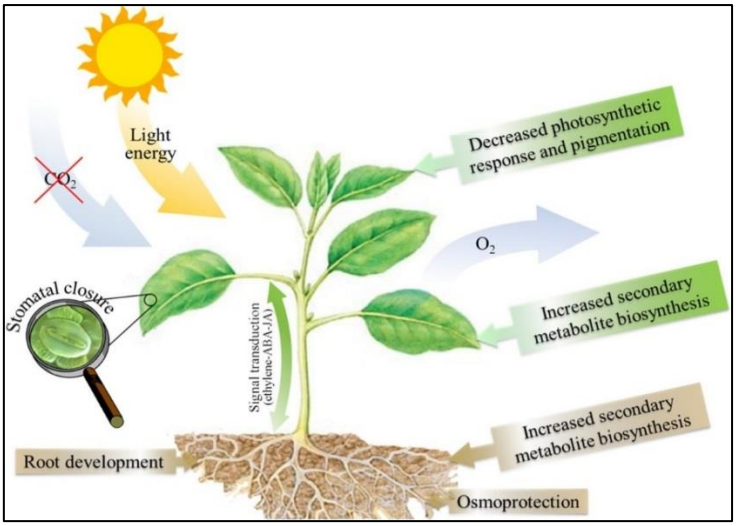


Figure 2.2: Plants response to cope with water deficit and high temperatures (Zingaretti *et al*., 2013).

2.5.1.3 Light

Light plays an important role in both dormancy stimulation as well as other physiological processes including photosynthesis and respiration. The amount and duration (photoperiod) play a huge role in how a seed germinates and grows (Hartmann *et al*., 2011).

Aufhammer *et al*. (1998) did a lot of research on how light effects seed germination of Amaranth. This research proved that light plays an important role in breaking seed dormancy (Dupriez & Leener, 1989). Flavonoids confer UV protection to plant tissues, and their accumulation due to UV-exposure is well documented (Stern, 2006).

2.5.2 Biotic factors

Biotic stress is defined as stress caused by living (biotic) factors which include all the other organisms within the habitat with which the affected plant interact (Stern, 2006). Plants possess innate immunity and therefore rely on defence response mechanisms for their protection (Odjakova & Hadjiivanova, 2001). The activation of the defence response is similar for all invaders including pathogens, bacteria and herbivore attacks (Walling, 2000). Plants have the ability to detect the presence of microorganisms and herbivores through an intricate surveillance system that recognises either microbial-associated molecular patterns (MAMPs) or pathogen-

associated molecular patterns (PAMPs), mechanical damage caused by abiotic or biotic wounding or the presence of elicitors secreted by phytophagous insects into host cells (Dangl & Jones, 2001; Brunissen *et al.,* 2009). The perception of a pathogenic or insect threat is a prerequisite for the activation of a defence response by a host plant.

2.5.2.1 Pathogens

Bacteria, viruses or any other micro-organism that can cause disease are known as pathogens (Oxford dictionary, 1991). Pathogens to crop yield and therefore have a great impact on the economics in the form of agricultural loss.

Among the many strategies available to disease, genetic resistance is the most cost effective and environment friendly (Agrios, 1988; Cornelissen & Melchers, 1993). Genes that confer resistance to pathogens have been clearly defined by conventional genetics and have been imported in the development of cultivars of almost all crops (Flor, 1971).

2.5.2.2 Herbivores

Herbivores refer to any animal or insect that feeds on plants (Oxford, 1991). Mechanical damage is caused by feeding upon a plant, when a plant is fed upon, the plant will start producing chemicals to survive by deterring the animal or insect from eating it. Different plant species produce different combinations, types and concentrations of chemical compounds (Stern, 2006).Tannins are well known and causes the plant to taste bitter when fed on. The compounds that are produced can be toxic (Stern, 2006).

The problem with insects feeding on plants is that the insect can introduce fungi or bacteria right into the plants inner cells (Stern, 2006). Herbivores can cause massive damage to crops either by feeding on exposed plants or by trampling the plants.

2.6 Vegetables

2.6.1 **Pepper** (*Capsicum annuum* L.)

Peppers are widely used in salads throughout the world and are from the Solanaceae family (Ballard *et al*., 1970). These peppers are green, yellow, orange and red, depending on their maturity. Peppers are also used as dips and in some gourmet meals. Peppers are native to Mexico, Central America and northern South America. Today China is the world largest producer of these peppers. Peppers have a rich content of antioxidants and vitamin C (DAFF, 2013). The level of carotene, like lycopene, is nine times higher in red peppers. Red peppers have twice the vitamin C content of green peppers (University of the District of Columbia).

2.6.2 **Tomato** (*Solanum lycopersicum* L.)

Tomatoes are edible fruits from the Solanaceae family, which originates from the South American Andes and spread throughout the world after the Americas were colonized by the Spanish (Flippone, 2016). Lycopene is a major constituent of tomatoes with many beneficial health properties for humans (Cohen, 2002; Rao *et al*., 2006). More than hundred and sixty million tonnes of tomatoes were produced worldwide in 2012, with China as the leading production country (Garming *et al*., 2014). This has a large impact on the economy, thus if there is a loss in production due to allelopathy, it could have an adverse impact on agriculture.

2.6.3 **Lettuce** (*Lactuca sativa* L.)

Lettuce is an annual plant of the daisy family Asteraceae and is often grown as a leafy vegetable (Acton, 2013) This vegetable was first cultivated by the ancient Egyptians who cultivate it from a weed whose seeds were rich in oil, into a food plant grown for its succulent leaves (Katz & Weaver, 2003). Lettuce spread throughout the world by the late 1900's. This vegetable is a good source of vitamin A, vitamin K, potassium, beta-carotene (found in darker green lettuces) and is also a good source of folate and iron (University of Illinois Extension, 2012). Because of the sesquiterpene lactones in lettuce, it has mild narcotic properties (Katz & Weaver, 2003). Lettuce is also used for religious and medical functions apart from being used as a leafy vegetable (Watts, 2007).

2.6.4 **Cucumber** (*Cucumis sativus* L.)

This vegetable is part of the Cucurbitaceae and originates from Southern Asia but has now spread to most continents (Renner *et al*., 2007). Cucumbers consist of mostly water, but vitamin K and B is noteworthy, copper, potassium, and manganese (USDA nutrient database). Cucumbers can help you to avoid nutrient deficiencies that are widespread among those eating a typical American diet. Cucumbers contain unique polyphenols and other compounds that may help reduce risk of chronic diseases (Mercola, 2014).

From literature, it is clear that there is a lack of information and knowledge concerning the active compounds synthesised by *A. cruentus* under temperature stress conditions. It is also unknown whether polar or non-polar compounds or a combination of the two, are responsible for allelopathic properties of *A. cruentus.* There is no information available on the allelopathic properties of stressed *A. cruentus* in crop rotation systems.

References

Acton, Q.A. 2013. Advances in Asteraceae Research and Application: 2013 Edition: Scholarly Brief, published by Scholarly Editions™, Atlanta, Georgia, USA. Pp 109.

Agrios, G.N. 1988. Plant pathology. 3rd ed. Academic Press, San Diego. Pp 500.

Allelopathic Journal. 2006. Welcome to the world of allelopathy. Allelopathy. Available at: www.allelopathy-journal.com. (17/09/13)

Allemann, J., Denner, F.D.N. 2006. Allelopathic influence of soils planted to *Amaranthus cruentus* L. on two tomato (*Lycopersicon esculentum* Mill.) cultivars. *S*. *A*. *J*. *Plant Soil*. **23**(2): 142-143.

Alvarez-Jubete, L., Wijngaard, H., Arendt, E.K., Gallagher, E. 2010. Polyphenol composition and *in vitro* antioxidant activity of amaranth, quinoa buckwheat and wheat as affected by sprouting and baking. *Food Chem*. **119**: 770-778.

Amini, R., An, M., Pratley, J., Azimi, S. 2009. Allelopathic assessment of annual ryegrass (*Lolium rigidum*): Bioassays. *Allelopathy J*. **24**(1): 67-79.

Amini, R., Movahedpour,F., Ghassemi-Golezani, K., Mohammadi-Nasab. A.D., Zafarani-Moattar, P. 2012. Allelopathic assessment of common amaranth by ECAM. *Int*. *Res*. *J*. *Appl*. *Basic Sci*. **3**(11): 2268-2272.

Amini, R., Ghanepour, S. 2013. Growth and yield of different types of dry bean affected by smooth amaranth (*Amaranthus hybridus* L.) shoot extracts. *Int*. *J*. *Agri*. *Crop Sci*. **5.2**: 115-119.

Amini, R., Pezhgan, H., Mohammodinasab, A.D., Shakiba, M.R. 2013. Growth analysis of eight common bean cultivars affected by weed interference. *Tech*. *J*. *Engin*. *Appl*. *Sci*. **3**(18): 2303-2308.

Appel, H.M. 1993. Phenolics in Ecological Interactions: The Importance of Oxidation. *J. Chem. Ecol*. **19**: 1521-1552.

Aufhammer W., Czuczorova D., Kaul H.P., Kruse M. 1998. Germination of grain amaranth (*Amaranthus hypochondriacus* × *A. hybridus*): effects of seed quality, temperature, light, and pesticides. *Eur. J. Agron*. **8:** 127-135.

Ayo, J.A. 2001. The effect of amaranth grain flour on the quality of bread. *Int*. *J*. *Food Prop*. **4**(2): 341-351.

Ayoola, G.A., Coker, H.A.B., Adesegun, S.A., Adepoju-Bello, A.A., Obaweya, K., Ezennia, E.C., Atangbayila, T.O. 2008. Phytochemical Screening and Antioxidant Activities of Some Selected Medicinal Plants Used for Malaria Therapy in Southwestern Nigeria. *Trop*. *J*. *Pharm*. *Res*. **7**(3): 1019-1024.

Azhar, N., Hussain, B., Ashraf, M.Y., Abbasi, K.Y. 2011. Water stress mediated changes in growth, physiology and. secondary metabolites of desi ajwain (*Trachyspermum ammi* L.). *Pakistan J. Bot*. **43**(SI):15-19.

Baghestani, A., Lemieux, C., Leroux, G.D., Baziramakenga, R., Simard, R.R. 1999. Determination of allelochemicals in sprig cereal cultivars of different competitiveness. *Weed Sci*. **47**: 498-504.

Balasundram, N., Sundram, K., Samman, S. 2006. Phenolic compounds in plant and agri-industrial byproducts: antioxidant activity, occurrence, and potential uses. *J. Food Chem*. **99**: 191-203.

Ballard, R.E., McClure, J.W., Eshbaugh, W.H., Wilson, K.G. 1970. A Chemosystematic Study of Selected Taxa of Capsicum. *Am. J. Bot*. **57**(2): 225–233.

Banerji, N. 1978. Chemical constituents of *Amaranthus spinosus* roots. *India J*. *Chem*. **17B**: 180-181.

Baquar, S.R. 1989. Medical and poisonous Plants of Pakistan, Printers, Karachi. Pp 506.

Barbehenn, R.V., Constabel, P.C. 2011. Tannins in plant-herbivore interactions. *Phytochem.* **72**:1551-65

Bavec, F., Bavec, M. 2006. Organic Production and Use of Alternative Crops. CRC Press, Taylor and Francis. Pp 241.

Becker, R., Wheeler, E.L., Lorenz, K., Stafford, A.E., Grosjean, O.K., Betschart, A.A., Saunders, R.M., Saunders, R.M. 1981. A composition study of amaranth grain. *J*. *Food Sci*. **46**: 1175-1180.

Bensch, C.N., Horak, M.J., Peterson, D. 2003. Interference of redroot pigweed (*Amaranthus retroflexus*), Palmer amaranth (*A. palmeri*), and common waterhemp (*A. rudis*) in soybean. *Weed Sci*. **51**: 37-43.

Bermejo, J.E., Leon, J. 1994. Neglected crops: 1492 from a different perspective. Rome: Plant production series No **26**: 93-10.

Berry, J.O. 2001. Kranz Anatomy and the C4 Pathway. Encyclopedia of life sciences. Pp 1-4.

Bertin, C., Yang, X., Weston, L.A. 2003. The role of root exudates and allelochemicals in the rhizosphere. *Plant Soil.* **256**: 67-83.

Bhadoria, P.B.S. 2011. Allelopathy: A natural way towards weed management. *Am*. *J*. *Exp*. *Agri.* **1**(1): 7-20.

Bhattacharya, A., Bhaumik, A., Rani, P.U., Mandal, S., Epid, T.T. 2010. Nano-particles – A recent approach to insect pest control. *Afri. J. Biotechnol*. **9**(24): 3489-3493.

Bhattachrjee, S. 2005. Reactive oxygen species and oxidative burst: roles in stress senescence and signal transduction in plant. *Curr*. *Sci*. **89**: 1113-1121.

Biopurify Phytochemicals Ltd, 2015. Alpha-Spinasterol. www.phytopurify.com. (18/04/2016).

Bishop, G.J., Koncz, C. 2002. Brassinosteroids and plant steroid hormone signalling. *Plant Cell*. **14**: S97-S110.

Bishop, G.J., Yokota, T. 2001. Plants steroid hormones, brassinosteroids: current highlights of molecular aspects on their synthesis/metabolism, transport, perception and response. *Plant Cell Physiol*. **42**(2): 114-120.

Björkman, O., Badger, M. R., Armond, P. A. 1980 Response and adaptation of photosynthesis to high temperatures. In *Adaptation of Plants to Water and High Temperatures Stress*, N. C. Turner and P. J. Kramer, eds., Wiley, New York. Pp 233–249.

Britton, G. 1991. Carotenoids, in Terpenoids (Charlwood, B.V., Banthorpe, D.B. eds) Chapter 7: Methods of Plant Biochemistry. Academic Press, London. **7**: 473-518.

Brown, W.V. 1975. Variations in anatomy, associations, and origins of Kranz tissue. *Am. J. Bot*. **62**: 395–402.

Brown, R.H., Hattersley, P.W. 1989. Leaf anatomy of C3–C4 species as related to evolution of C4 photosynthesis*. Plant Physiol*. **91**: 1543–1550.

Brown, R. 1995. Prejudice: Its Social Psychology. Pp 319.

Brown, M.J., Ferruzzi, M.G., Nguyen, M.L., Cooper, D.A., Eldridge, A.L., Schwartz, S.J., White. W.S. 2004. Carotenoid bioavailability is higher from salads ingested with full-fat than with fat-reduced salad dressings as measured with electrochemical detection. *Am. J. Clin. Nutr.* **80**(2): 396-403.

Brunissen, L., Cherqui, A., Pelletier, Y., Vincent, C., Giordanengo, P. 2009. Host-plant mediated interactions between two aphid species. *Emtomol*. *Exper*. *Appl*. **132**: 30-38.

Cai, Y., Sun, M., Corke, H. 1998. Colorant Properties and Stability of *Amaranthus* Betacyanin Pigments. *J. Agric. Food Chem*. **46**(11): 4491-4495.

Cai, Y., Sun, M., Corke, H. 2005. Caracterization and application of betalain pigments from plants of the Amaranthaceae. *Trends Food Technol.* **16**: 370-376.

Castro P.R.C., Kluge R.A., Peres L.E.P. 2005. Manual de fisiologia vegetal: teoria e peática. *Agronémica Ceres: Piracicaba.* Pp 650.

Chen, Y., Guo, Q., Liu, L., Liao, L., Zhu, Z. 2011. Influence of fertilization and drought stress on the growth and production of secondary metabolites in *Prunella vulgaris* L. *J*. *Medi*. *Plants Res*. **5**(9): 1749-1755.

Cheng, A.X., Lou, Y.G., Mao, Y.B., Lu, S., Wang, L.J., Chan, X.Y. 2007. Plant terpenoids: biosynthesis and ecological functions. *J*. *Integr*. *Plant Bio*. **49**: 179-186.

Chevallier, A. 1996. The Encyclopedia of Medical Plants. Publisher Pub, D.K (1996). Pp 336.

Chopra, R.N., Nayar, S.L., Chopra, I.C., Asolkar, L.V., Kakkar, K.K. 1986. Glossary of Indian medical plants; [with] Supplement. *New Delhi: Council of Scientific & Industrial Research.* Pp 1956-1992.

Claro de Souza, M., de Cavalho, H.B., da Costa Aguiar Alves, P.L., Giancotti, P.R.F. 2011. Allelopathy in pigweed (A review). *Comm*. *Plant Sci*. **1**: 5-12.

Coastea, M., Demason, D.A. 2001. Stem morphology and anatomy in *Amaranthus* L. (Amaranthacea) Taxonomic Significance. *Terry Bot*. **128**(3): 254-281.

Connick, W.J., Bradow, J.M., Legendre, M.G. 1989. Identification and Bioactivity of Volatile Allelochemicals from Amaranth Residues. *J. Agric. Food Chem.* **37**: 792-796.

Cohen L. 2002. A review of animal model studies of tomato carotenoids, lycopene, and cancer chemoprevention. *Exp Biol Med*. **227**(10): 864–868.

Craft, N.E., Soares, J.H. 1992. Relative solubility, stability, and absorptivity of lutein and .beta.-carotene in organic solvents. *J*. *Agri*. *Food Chem*. **40**(3): 431-434.

Colquhoum, J.B. 2006. Allelopathy in weeds and crops: myths and facts. *Process of the 2006 Wisconsin Fertilizer, Aglime & Pest Management Conference*. **45**: 318-320.

Cornelissen, B.J., Melchers, L.S. 1993. Strategies for control of fungal diseases with transgenic plants. *Plant Physiol*. **101**: 709-712.

Cunningham, S. 2010. Growing Amaranthus – Callalou, Laf Sag, Lalshank, Tangerio. Sowing New Seed Project. UK. Pp 3.

D'Abrosca, B., De Maria, P., DellaGreca, M., Fiorentino, A., Golino, A., Izzo, A., Monaco, P. 2006. Amarantholidols and amarantholidosides: new nerolidol derivatives from the weed *Amaranthus retroflexus*. *Tetrahedron.* **62**: 640-646.

Dangl, J.L., Jones, J.D. 2001. Plant pathogens and integrated defence responses to infection. *Nature*. **411**(6839): 826-833.

de Albuquerque, M.B., das Santos, R.C., Lima, L.M., Filho, P.A.M., Nogueria, R.J.M.C., Da Câmara, C.A.G., Ramos, A.R. 2010. Allelopathy, an alternative tool to improve cropping systems. A review. *Agro*. *Sustain*. *Develop*. **31**: 379-395.

De Oliveira P.R.G., Rodriquez-Amaya D.B. 2007. Processed and Prepared Corn Products as Sources of Lutein and Zeaxanthin: Compositional Variation in the Food Chain. *J*. *Food Sci*. **72**(1): S79-S85.

Department of Agriculture, Forestry and Fisheries. 2010. Amaranthus: Production guideline. *DAFF.* Pp 1-14.

Department of Agriculture, Forestry and Fisheries. 2013. Production Guideline: Sweet pepper (*Capsicum annuum*). *Plant Prod*. Pp 1-20.

Dhellot, R.J., Matouba, E., Maloumbi, M.G., Nzikou, J.M., Safou. D.G., Linder, M., Desobry, S., Parmentier, M. 2006. Extraction, chemical composition and nutritional characterization of vegetable oils: Case of *Amaranthus hybridus* (var 1 and 2) of Congo Brazzaville. *Afri. J .Biotechnol*. **5**: 1095-1102.

Dhole, J.A., Lone, K.D., Dhole, G.A., Bodke, S.S. 2013. Allelopathic effect of aqueous and ethanolic extracts of some common weeds on seed health of *Triticum aestivum* L. (Wheat). *Int*. *J*. *Curr*. *Mircobiol. Appl*. *Sci*. **2**(6): 254-260.

Di Mascio, P., Kaiser, S., Sies, H. 1989. Lycopene as the most efficient biological carotenoid singlet oxygen quencher. *Arch. Biochem. Biophys*. **274**: 532-538.

Ding, J., Sun, Y., Xiao, C.L., Shi, K., Zhou, Y.H., Yu, J.Q. 2007. Physiological basis of different allelopathic reactions of cucumber and figleaf gourd plants to cinnamic acid. *J. Exper. Bot*. **58**(13): 3765-3773.

Duke, J.A., Ayensu, E.S. 2008. Medical Plants of China. *J*. *Bot*. *Taxon. Geobot*. **98**(7-8): 398.

Dupriez, H., Leener, D.E. 1989. African Gardens and Orchards: Growing Vegetables and Fruits. Macmillians, London. Pp 282-287.

Ebert, A.W., Wu, E., Wang, S. 2011. Vegetable amaranth (*Amaranthus* L.). International Co-operators' Guide. AVRDC-The World Vegetable Centre. **11**:1-8.

Ehleringer, J. 1983. Ecophysiology of *Amaranthus palmeri*, a Sonoran desert summer annual. *Oecologia* (Berlin). **57**: 107-112.

Faravanim, Baki, H.B., Khalijah, A. 2008. Assessment of allelopathic potential of *Melastomamalabathricum* L. on *Radish raphanussativus* L. and Barnyard Grass (*Echinochloacrus-galli*). *Notulae Botanicae Horti Agrobotanici Cluj-Napoca*. **36**(2): 54-60.

Fares, S., Oksanen, E., Lännenpää, M., Julkunen-Tiitto, R., Loreto, F. 2010. Volatile Emissions and Phenolic Compound Concentrations along a Vertical Profile of *Populus nigra* Leaves Exposed to Realistic Ozone Concentrations. *Photo*syn. *Res*. **104**: 61-74

Fasuyi, A.O. 2006. Nutritional potentials of some tropical vegetable leaf meals: chemical characterization and functional properties. *Afri. J.Biotechnol*. **5**: 49-53.

Fasuyi, A.O. 2007. Bio-nutritional evaluations of three tropical leaf vegetables (*Telfairia occidentalis, Amaranthus cruentus* and *Talinum triangulare*) as sole dietary protein sources in rat assay. *Food Chem.* **103**: 757-765.

Ferguson, J.J., Rathinasabapathi, B., Chase, C.A. 2003. Allelopathy: How Plants Suppress Other Plants. University of Florida. IFAS Extension. Pp 5.

Ferreira, M.I., Reinhardt, C.F. 2010. Field assessment of crop residues for allelopathic effects on both crops and weeds. *Agron. J*. **102**: 1593-1600.

Flippone, P.T. 2016. Tomato History – "The history of tomatoes as food". Home cooking. www.foodhistory/a/tomatohistory/htm. Retrieved 18/05/2016.

Flor, A.H. 1971. Current status of the gene-for-gene concept. *Annu. Rev. Phytopathol*. **9**: 275-296.

Fomsgaard, I.S., Añon, M.C., Barba de la Rosa, A.P., Christophersen, C., Dusek, K., Délano-Frier, J., Pérez, J.E., Fonseca, A., Janovská, D., Kudsk, P., Labouriau, R.S., Romero, M.L.L., Martínez, N., Matus, F., Matusová, K., Mathiassen, S.K., Noellemeyer, E.J., Pedersen, H.A., Stavelikova, H., Steffensen, S.K., de Troiani, R.M., Taberner, A. 2010. Amaranth: Future-Food. AARHUS UNIVERSITY Aarhus University, Faculty of Agricultural Sciences, Department of Integrated Pest Management, Denmark. ISBN: 978-87-91949-62-3.

Foster, S., Duke, J.A. 1990. A Field Guide to Medical Plants. Eastern and Central N. America. *Houghton Mifflin Co*. ISBN 0395467225.

Gallie, D.R. 2013. L-Ascorbic acid: a multifunctional molecule supporting plant growth and development. *Scientifica*. **2013**: 1-24.

Gamel, T.H., Mesallam, A.S., Damir, A.A., Shekib, L.A.. Linssen, J.P. 2007. Characterization of Amaranth Seed Oils. *J. Food Lipids*. **14**: 323-334.

Garming, H., Strohm, K., Dirkmeyer, W. 2014. Tomato – A new crop in *agri benchmark* Horticulture. Thünen Institute of Farm Economics. Pp 24.

Gawronska, H., Golisz, A. 2006. Chapter 10: Allelopathy and Biotic Stress. Allelopathy: A Physiological Process with Ecological Implications. Springer, Netherlands. Pp 211-227.

Ghafarbi, S.P., Hassannejad, S., Lotfi, R. 2012. Allelopathic effects of wheat seed extracts on seed and seedling growth of eight selected weed species. *Int. J. Agri. Crop Sci*. **4**(19): 1452-1457.

Gobbo-Neto, L., Lopes, N.P. 2007. Medicinal plants: factors of influence on the content of secondary metabolites. *Quimia*. *Nova*. **30**(2): 374.

González, R., Tosi, E., Ré, E., Añón, M.C., Pilosof, A.M.R., Martinez, K. 2007. Amaranth starch-rich fraction properties modified by high-temperature heating. *Food Chem.* **103:** 927–934.

Goralczyk, R. 2009. Beta-carotene and lung cancer in smokers: review of hypotheses and status of research. *Nutr*. *Cancer*. **61**: 767-774.

Gouvea, D.R., Meloni, F., Ribeiro, A.B.B., Lopes, J.L.C., Lopes, N.P. 2012. A new HPLC-DAD-MS/MS method for the simultaneous determination of major compounds in the crude extract of *Lychnophora salicifolia* Mart. (Brazilian arnicão) leaves: Application of chemical variability evaluation. *Analytia Chimica Acta*. **748**: 28-36.

Govindarajan, R., Vijayakumar, M., Pushpangadan, P. 2005. Antioxidant approach to disease management and the role of 'Rasayana' herbs of Ayurveda. *J*. *Ethnopharmacol*. **99**: 165-178.

Graber, K.H. 2014. Amaranth-May Grain of the Month. Whole Grain Council. Pp 3.

Graβmann, J. 2005. Terpenoids as plant antioxidants. In: Litwack, G., ed. *Plant Hormones*. San Diego, CA, USA: Elsevier Academic Press. Pp 505-535.

Grieve, R.A.F. 1984. The impace cratering rate in recent time. *J*. *Geophys*. *Res*. **89**: 148-227.

Grobelnik, S.G., Turinek, M., Jakop, M., Bavec, M., Bavec, F. 2009. Nutrition value and use of grain amaranth: potential future application in bread making. *Agricutlura*. **6**:43-53.

Gross, J. 1987. Pigments in fruits. Academic Press, London. Pp 1.

Gross, J. 1999. Pigments in vegetables. Chlorophylls and carotenoids. Avi:Van Nostrand Reinhold Company Inc, New York. Pp 147.

Grosz-Heilmann, R., Golz, J.T., Helgeson, D.L. 1990. Amaranth: A food crop from the past for the future. Pp 20.

Guerra-Matias, A.C., Arêas J.A.G. 2005. Glycemic and insulinemic responses in women consuming extruded amaranth (*Amaranthus cruentus* L.). *Nutr. Res.* **25**: 815-822.

Guo, P., Al-Khatib, K. 2003. Temperature effects on germination and growth of redroot pigweed (*Amaranthus retroflexus*), Palmer amaranth (*A. plameri*), and common waterhemp (*A. rudis*). *Weed Sci*. **51**: 869-875.

Hartmann, H.T., Kester, D.E., JR, F.T.D., Geneve, R.L. 2011. Plant propagation principles and practices, 7th Edition. Prentice Hall Publishers, New Jersey. Pp 281-340.

Hasanuzzaman, M. 2014. Allelopathy. Department of Agronomy Sher-e-Bangla Agricultural University. Chapter 6. Pp 1-5.

Heim, K.E., Tagliaferro, A.R., Bobilya, D.J. 2002. Flavonoid antioxidants: Chemistry, metabolism and structure – activity relationships. *J*. *Nut*. *Biochem*. **13**: 572-584.

Heisey, R.M. 1990. Evidence for Allelopathy by Tree-of Heaven (*Ailanthus altissima*). *J. Chem. Ecol*. **16**(6): 2039-2055.

Heisey, R.M. 1997. Allelopathy and the Secret Life of *Ailanthus altissima*. *Arnoldia*. **57**(3): 28-36.

Hilou, A. 2006. *Etude phytochimique et activités biologiques d'extraits de deux Caryophyllales à bétalaïnes;* Amaranthus spinosus *L. (Amaranthaceae) et* Boerhaavia erecta *(Nyctagynaceae),plantes médicinales du Burkina Faso*. Thèse unique de doctorat, Université de Ouagadougou. Pp 176.

Hoiberg, D. 2016. Amaranth family. Plant family: Amaranthaceae. Encyclopaedia Britannica. global.britannica.com/plant/Amaranthaceae#ref230955.

Hunter, K.J., Fletcher, J.M. 2002. The antioxidant activity and composition of fresh, frozen, jarred and canned vegetables. *Inno*. *Food Sci*. *Emerg*. *Technol.* **3**(4): 399-409.

Hura, T., Hura, K., Grzesiak, S. 2009. Possible contribution of cell-wall-bound ferulic acid in drought resistance and recovery in triticale seedlings. *J*. *Plant Physiolo*. **166**(16): 1720-1733.

Inderjit, D., K. M. M., Einhellig, F. A., eds. 1995. *Allelopathy: Organisms, Processes, and Applications*. ACS Symposium series American Chemical Society, Washington, DC.

International Development Reseach Centre. 2016. Facts and Figures on Food Biodiversity. www.idrc.com. Retrived: 20/10/2016.

Kachiguma, N,A., Mwase, W., Maliro, M., Damaliphetsa, A. 2015. Chemical and Mineral Composition of Amaranth (*Amaranthus* L.) Species Collected From Central Malawi. J. *Food Res*. **4**(4): 92-102.

Katz, S.H., Weaver, W.W. 2003. Encyclopedia of Food and Culture. Publisher: Scribner. New York. Pp 375–377.

Kauffman, C.S., Weber, L.E. 1990. Grain amaranth. In: Advances in New Crops, J. Janick & J.E. Simon (Eds). Timber Press, Portland, Oregon: Pp 127-139.

Kawanishi, S., Hiraku, Y., Oikawa, S. 2001. Mechanism of guanine-specific DNA damage by oxidative stress and its role in carcinogenesis and aging. *Muta*. *Res*. **488**: 65-76.

Keeley, P.E., Carter, C.H., Thullen, R.J. 1987. Influence of Planting Date on Growth of Palmer Amaranth (*Amaranthus palmeri*). *Weed Sci*. **35**(2): 199-204.

Khanh, T.D., Chung, I.M., Tawata, S., Xuan, T.D. 2006. Weed Suppression by Passifloraedulis and its Potential Allelochemicals. *Weed Res*. **46**: 296-303.

Kim, K.U., Shin, D.H. 2003. The importance of allelopathy in breeding new cutivars. In *Weed Mang*. *Develop*. *Coun*. FAO Plant Production and Protection Paper No. 120. Ed. Labrada, R., editor. Rome: *Food Agri*. *Org*. Pp 195-210.

Kimura M., Rodriguez-Amaya D.B. 2003. Carotenoid Composition of Hydroponic Leafy Vegetables. *J*. *Agri*. *Food Che*. **51**(9): 2603-2607.

Kraujalis, P., Venskutonis, P.R., Kraujaliene, V., Pukalskas, A. 2013. Antioxidant Properties and Preliminary Evaluation of Phytochemical Composition of Different Anatomical Parts of Amaranth. *Plant Foods Hum Nutr*. **68**: 322‑328.

Krinsky N.I. 1993. Actions of Carotenoids in Biological Systems. *Annu*. *Rev*. *Nutr*. **13**: 561-587.

Konstantinović, B., Blagojević, M., Konstantinivić, B., Samardžić,N. 2014. Allelopathic effect of weed species *Amaranthus retroflexus* L. on maize seed germination. *Romanian Agri*. *Res*. **31**: 315-321.

Letournea, O., Failes, G.D., Heggenes, H.G. 1956. The Effect of Aqueous Extracts of Plant Tissue on Germination of Seeds and Growth of Seedlings. *Weeds*. **4**: 363-368.

Levitt, J. 1980. *Responses of plants to environmental stresses,* Vol. 1, 2nd ed. Academic Press, New York. Pp 698.

Li, J., Ou-Lee, T.M., Raba, R., Amundsom, R.G., Last, R.L. 1993. Arabidopsis Flavonoid Mutants Are Hypersensitive to UV-B Irradiation. *Plant Cell*. **5**(2): 171-179.

Li, Z.H., Wang, Q., Ruan, X., Pan, C.D., Jiang, D.A. 2010. Phenolics and Plant Allelopathy. *Molecules*. **15**: 8935-8952.

Lin, L.Z., Zhou, D.H., Zheng, X.T. 2005. Effects of traditional Chinese medicine in improving quality of life of patients with non-small cell lung cancer in late stage. *Chinese J*. *Inte*. *Trad*. *Western Medi*. **26**(5): 389-393.

Loreto, F., Förster, A., Dürr, M., Csiky, O., Seufert, G. 1998. On the Monoterpene Emission under Heat Stress and on the Increased Thermo-tolerance of Leaves of *Quercus ilex* L. Fumigated with Selected Monoterpenes. *Plant Cell Environ*. **21**: 101-107.

Loreto, F., Pinelli, P., Manes, F., Kollist, H. 2004. Impact of Ozone on Monoterpene Emissions and Evidences for an Isoprene-Like Antioxidant Action of Monoterpenes Emitted by *Quercus ilex* (L.) Leaves. *Tree Physiol*. **24**: 361-367.

Maiyo, Z.C., Ngure, R.M., Matasyoh, J.C., Chepkorir, R. 2010. Phytochemical constituents and antimicrobial activity of leaf extracts of three *Amaranthus* plant species. *Afri*. *J*. *Biotechnol.* **9**(21): 3178-3182.

Massinga, R.A., Currie, R.S., Horak, M.J., Boyer Jr, J. 2001. Interference of Palmer amaranth in corn. *Weed Sci*. **49**(2): 202-208.

McLauren D.S., Frigg M. 2002. Practical Guide on Vitamin A in Health and Disease, Sight and Life Manual, Sight and Life Publication. Pp 172.

Menges, R.M. 1988. Allelopathic effect of Palmer amaranth (*Amaranthus palmeri*) on seedling growth. *Weed Sci*. **36**: 325-328.

Mensah, J.K., Okoli, R.I., Ohaju-Obodo, J.O., Eifediyi, K. 2008. Phytochemical, nutritional and medical properties of some leafy vegetables consumed by Edo people of Nigeria. *Afri. J. Biotechnol.* **7**(14): 2304-2309.

Mercola, J. 2014. 9 Health benefits of Cucumbers. www.Mercola.com. Retrieved 18/05/2016.

Miller, D.A. 1996. Allelopathy in forage crop systems. *Agron*. *J*. **88**: 854-859.

Minorsky, P.V. 2002. Allelopathy and Grain Production. *Plant Physiol*. **130**(4): 1745-1746.

Mittler, R., Vanderauwera, S., Gollery, M., Van Breusegem, F. 2004. Reactive oxygen gene network of plants. *Trends Plant Sci*. **9**: 490-498.

Mlakar, S.G., Jakop, M., Bavec, M., Bavec, F. 2012. Allelopathic effects of *Amaranthus retroflexus*and *Amaranthus cruentus* extracts on germination of garden cress. *Afri. J. Agri. Res*. **7**(10): 1492-1497.

Moerman, D.E. 1998. Native American Ethnobotany. ISBN: 0881924539, 9780881924534. Pp 927.

Mosyakin, S.L., Robertson, K.R. 1996. New infrageneric taxa and combinations in *Amaranthus* (Amaranthaceae). *Annales Botanici Fennici*. **33**: 275-281.

Mroczek, A., 2015. Phytochemistry and bioactivity of triterpene saponins from Amaranthaceae family. *Phytochem*. *Rev*. DOI 10.1007/s11101-015-9394-4.

Mshelia, J.S., Degri, M.M. 2014. Effect of different levels of poultry manure on the performance of Amaranthus (*Amaranthus caudatus*L.) in Bama, Nigeria. *Int*. *J*. *Sci*. *Nat*. **5**(1):121-125.

Mwangi, D.K. 1993. Grain amaranth as a potential food and subsistence farming in Kenya. Kenyatta University, Nairobi. Pp 1-50.

Myers, R.L. 1996. Amaranth: New crop opportunity. In:J.Janick (ed.), Progress in new crops. ASHS Press, Alexandria, VA. Pp 207-220.

Myers, R.L. 1998. Nitrogen fertilizer effect on graim amaranth. *Agron*. *J*. **90**: 597-602.

Nacoulma, O.G. 1996. *Plantes médicinales et pratiques médicales traditionnelles au Burkina Faso: cas du plateau central*; Thèse d'état, Université de Ouagadougou. Pp 574.

Nakashima, A., Mori, K., Kaneko, Y.S., Hayashi, N., Nagatsu, T., Ota, A. 2011. Phosphorylation of the N-terminal portion of tyrosine hydroxylase triggers proteasomal digestion of the enzyme. *Biochem*. *Biophysi*. *Res*. *Comm*. **407**(2): 343-347.

National Research Council. 1984. Amaranth: Modern Prospects for an Ancient Crop. National Academy Press, Washington, D.C.

Nevens, F., Reheul, D. 2001. Crop rotation versus monoculture; yield, N yield, and ear fraction of silage maize at different levels of mineral N fertilization. *NJAS-Wagen*. *J*. *Life Sci.* **49**(4): 405-425.

New World Encyclopedia. 2009. Amaranth. Paragon House Publishers. Retrived: 29/05/2014.

Obaid, K.A., Qasem, J.R. 2005. Allelopathic activity of common weed species on vegetable crops grown in Jordan. Plant Protection Department, Faculty of Agriculture, University of Jordan, Amman, Jordan. Pp 1.

Oboh, G., Raddatz, H., Henle, T. 2008. Antioxidant properties of polar and non-polar extracts of some tropical green leafy vegetables. *J*. *Sci*. *Food Agri*. **88**:2486–2492.

Odhav, B., Beekrum, S., Akula, U., Baijnath, H. 2007. Preliminary assessment of nutritional value of traditional leafy vegetables in KwaZulu-Natal, South Africa. *J. Food Comp. Anal.* **20**: 430-435.

Odjakova, M., Hadjiivanova, C. 2001. The complexity of pathogen defence in plants. *Bulg J Plant Physiol*. **27**: 101-109.

Ogbe, R.J., Adoga, G.I., Abu, A.H. 2010. Antianaemic potentials of some plant extracts on phenyl hydrazine – induced amaemin in rabbit. *J*. *Medici. Plants Res*. **4**(8): 680-684.

Oloyede, F.M., Obyede, F.A., Obuotor, E.M. 2013. Effects of Plant Maturity of the Antioxidant Profile of *Amaranthus cruentus* L. and *Celosia argentea* L. *Bul*. *Environ, Pharam*. *Life Sci*. **2**: 18-21.

Olufemi, B.E., Assiak, I.E., Ayoade, G.O., Onigemo, M.A. 2003. Studies on the effect of *Amaranthus spinosus* leaf extract on the hematology of growing pigs. *Afri*. *J*. *Biomedi*. *Res*. **6**: 149-150.

Olumayokun, A., Olajid, B.R., Ogunleya, T., Erinle, O. 2004. Anti-inflammatory properties of *Amaranthus spinosus*. *Pharma*. *Biol*. Pp 521-525.

Omosun, G., Markson, A.A., Mbanasor, O. 2008. Growth and Anatomy of *Amaranthus Hybridus* as Affected by Different Crude Oil Concentrations. *American-Eurasian J*. *Sci*. *Res*. **3**(1): 70-74.

Oxford English Dictionary. 1991. Oxford University Press. ISBN 0-19-861069.

Ozsoy, S., Esel, E., Kula, M. 2009. Serum oxytocin levels in patients with depression and the effects of gender and antidepressant treatment. *Psychiatry Research*. **169**(3): 249-252.

Pamplona-Roger D. 1998. Encyclopedia of Medicinal Plants. Publisher: Saeliz, Spain. Pp 1-1204.

Peñuelas, J., Llusià, J. 2002. Linking Photorespiration, Monoterpenes and Thermo-tolerance in *Quercus*. *New Phytologist*. **155**: 227-237.

Polterait, O. 1997. Antioxidants and free-radical scavengers of Natural Origin. *Curr*. *Organic Chem*. **1**: 415-440.

Pratley, J.E. 1996. Allelopathy in annual grasses. *Plant Protec*. *Quar*. **11**: 213-214.

Pratley, J.E. 2006. Allelopathy- New weed control options for maize. Maize Association of Australia. 6th Triennial Conference. Pp 1-4.

Putnam, A.R., Tang, C.S. 1986. The Science of Allelopathy. ISBN 0-471-83027-5. Pp 317.

Putnam, A.R. 1988. Allelochemicals from Plants as Herbicides. *Weed Tech*. **2**(4): 510-518.

Qasem, J.R. 1995. The allelopathic effect of three *Amaranthus* spp. (pigweeds) on wheat (*Triticum durum*). *Weed Res*. **35**(1): 41-49.

Radosavljevic, M. 2006. Comparison of *Amaranthus cruentus* and *Zea mays*, L. starch characteristics. *Genetika*. **38**: 31-36.

Raheena, B. 2007. Food, Nutrition and Dietetics. 2nd Edition, Sterling Publisher Private Limited. Pp 106-109.

Rahimzadeh, F., Tobeh, A., Jamaati-e-Somarin, S. 2012. Study of allelopathic effects of aqueous extracts of roots and seeds of goosefoot, red-root amaranth and field bindweed on germination and growth on lentil seedlings. *Int*. *J. Agron*. *Plant Prod*. **3**(9): 318-326.

Raison, J. K., Pike, C. S., Berry, J. A. 1982. Growth temperature induced alterations in the thermotropic properties of *Nerium oleander* membrane lipids. *Plant Physiol.* **70**: 215–218.

Ramakrishna, A., Ravishankar, G.A. 2011. Influence of abiotic stress signals on secondary metabolites in plants. *Plant Signal Behav*. **6**(11): 1720-1731.

Rao, A.V., Ray, M.R., Rao, L.G. 2006. Lycopene. *Adv*. *Food Nutr*. *Res*. **51**: 99–164.

Rennenberg, H., Loreto, F., Polle, A., Brilli, F., Fares, S., Beniwal, R.S., Gessler, A. 2006. Physiological Responses of Forest Trees to Heat and Drought. *Plant Biol*. **8**: 556-571.

Renner, S.S., Schaefer, H., Kocyan, A. 2007. *"Phylogenetics of Cucumis (Cucurbitaceae): Cucumber (C. sativus) belongs in an Asian/Australian clade far from melon (C. melo)"*. *BMC Evol*. *Biol.* **7**: 58.

Ribaya-Mercado, J.D., Maramg, C.C., Tengco, L.W., Dolnikowski, G.G., Blumberg, J.B., Solon, F.S. 2007. Carotene-rich plant foods ingested with minimal dietary fat enhance the total-body vitamin A pool size in Filipino school children as assessed by stable-isotope-dilution methodology. *Am. J. Clin. Nutr.* **85**: 1041-1049.

Rice, E.L. 1984. Allelopathy. 2nd. Edn. Orlando, Florida, USA: Academic Press. Pp 67-68.

Rispail, N., Morris, P., Webb, K.J. 2005. Phenolic Compounds: Extraction and Analysis. Chapter 7.5 (In) *Lotus japonicus* Handbook. Pp 349-355.

Rivero, R.M., Ruiz, J.M., Garcia, P.C., López-Lefebre, L.L., Sánchez, E., Romero, L. 2001. Resistance to cold and heat stress: accumulated of phenolic compounds in tomato and watermelon plants. *Plant Sci*. **160**: 315-321.

Rodriguez-Amaya, D.B. 1993. Nature and distribution of carotenoids in foods. In Charalambous G (ed), Shelf-life studies of foods and beverages. Chemical, biological, physical and nutritional aspects. Elsevier Science Publishers, Amsterdam. Pp 547-589.

Rodriguez-Amaya, D.B. 1999. Changes in carotenoids during processing and storage of foods. *Arch*. *Latinoam Nutr*. **49**(3 Suppl 1): 38S-47S.

Ruskin, F.R. (ed). 1984. Amaranth, Modern prospects for an ancient crop. National Academy Press, Washington, D.C. Pp 1-3.

Russell, P.L. 2016. The Essential History of Mexico: From Pre-Conquest to Present. Taylor & Frances, New York. Pp 1-449.

Sadaqa, E.A., Bawazir, A.A., Qasem, J.R. 2010. Allelopathic activity of some common weeds species in onion fields. *Allelopathy J*. **26**(2): 175-184.

Samad, M.A., Rahman, M.M., Hossain, A.K.M.M., Rahman, M.S., Rahman, S.M. 2008. Allelopathic effects of five selected weed species on seed germination and seedling growth of corn. *J. Soil. Nature.* **2** (2):13-18.

Sauer, J. 1957. Recent migration and evolution of the dioecious Amaranths. *Evol*. **11**: 11-31.

Saunders, R., Becker, R. 1984. Amaranthus: a potential food and feed resource. *Adv*. *Cereal Sci*. *Technol*. **6**: 357-396.

Schiedt, K., Liaan-Jansen, S. 1995. Isolation and analysis. In: Carotenoids, Volume 1A: Isolation and Analysis (Britton, G., Liaan-Jansen, S.: Pfander, H., Eds.). Birkhäuser Verlag, Basal, Switzerland. Pp 81-108.

Schwartz, D.P. 1988. Improved methods for quantitating and obtaining the unsaponifiable matter of fats and oils. *J*. *Am*. *Oil Chem. Soc*. **65**: 246-252.

Sellers, B.A., Smeda, R.J., Johnson, W.G., Kendig, J.A., Ellersieck, M.R. 2003. Comparative growth of six *Amaranthus* species in Missouri. *Weed Sci*. **51**(3): 329-333.

Shah, A.H.A. 2005. Spinoside, new coumaroyl flavone glycoside from *Amaranthus spinosu*s. *Arc*. *Pharm*. *Res*. 27(12):1216-1219.

Shahrokhi, S., Hejazi, S.N., Khodabandeh, H., Faboodi, M., Faramarzi, A. 2011. Allelopathic Effects of Aqueous Extracts of Pigweed, *Amaranthus retroflexus* L.

Organs on Germination and Growth of Five Barley Cultivars. 3rd International Conference of Chemical, Biological and Environmental Engineering. IPCBEE, IACSIT Press, Singapore. Pp 80-84.

Sharma, H.C., Agarwal, R.A. 2011. Role of some chemical components and leaf hairs in varietal resistance in cotton to jassid, *Amrasca biguttula biguttula* Ishida. *J*. *Entomol*. *Res*. **7**:145-149.

Shettel, N.L., Balke, N.E. 1983. Plant growth response to several allelopathic chemicals. *Weed Sci. Soc. Amer*. Allen Press. JSTOR. Pp 293-298.

Shroyer, J.P., Stegmeier, W.D., Mikesell, M. 1990. Grain Amaranth. Co-operative Extension Service. Kansas State University. Manhattan. Pp 1-2.

Shukla, S., Bhargava, A., Chatterjee, A., Srivastava, A., Singh, S.P. 2005. Estimates of genetic variability in vegetable amaranth (*A. tricolor*) over different cuttings. *Hort.Sci* (Prague). **32**(2):60-67.

Smirnoff, N., Wheeler, G.L. 2000. Ascorbic acid in plants: biosynthesis and function. *Cri*. *Rev*. *Biochem*. *Mol*. *Biol*. **35**(4): 291-314.

Sotomayor, D.A., Lortie, C.J. 2014. Allelopathy. Oxford Bibliographies. Pp 2.

Stähelin, H.B., Gey, K.F., Eichholzer, M., Lüdin, E. 1991. β – Carotene and cancer prevention: the Besal study. *Am. J. Clin. Nutr.* **53**: 265S-269S.

Stallknecht, G.F., Schulz-Schaeffer, J.R. 1993. Amaranth Rediscovered. In: J.Janick and J.E. Simon (eds.), New crops. Wiley, New York. Pp 211-218.

Steckel, J. 2004. The dioecious Amaranth spp: Here to stay. *Weed Tech*. **21**:567-570.

Steckel, L.E. 2007. The Dioecious *Amaranthus* spp.: Here to Stay. *Weed Sci*. *Soc*. *Amer*. **21**(2): 567-570.

Stern, K.R. 2006. Introductory Plant Biology, tenth edition. McGraw Hill. New York. Pp 624.

Su, Q., Rowley, K.G., Itsiopoulos, C., O'Dea, K. 2002. Identification and quantitation of major carotenoids in selected components of the Mediterranean diet: green leafy vegetables, figs and olive oil. *Eur*. *J*. *Clin*. *Nutr*. **56**(11): 1149-54.

Sultana, S., Asaduzzaman, M.D., Sohail, A. 2012. Allelopathic potential of Amaranthus viridis L. on canola and annual ryegrass. *Bangladesh Res*. *Pub*. *J*. **7**(1): 29-35.

Taiz, L., Zeiger, E. 2012. Plant Physiology. 5th Edition. Sinauer Associates, Sunderland. Pp 559.

Tamer, H., Gamel, A.S., Mesallam, A.A, Damir, L.A., Josef, P.L. 2006. Characterization of amaranth seed oils. *J. Food Lipids*. **14**: 323-334.

Tanumihardjo S.A., 2008. Food-Based Approaches for Ensuring Adequate Vitamin A Nutrition, *Comprehensive Rev*. *Food Sci*. *Food Saf*. **7**: 373-381.

Teutonico, R.A., Knorr, D. 1985. Amaranth: Composition, properties, and applications of a rediscovered food crop. *Food Tech*. **39**: 49-60.

Tholl, D. 2015. Biosynthesis and biological functions of terpenoids in plants. *Adv*. *Bichem*. *Eng*. *Biotechnol*. Pp 148-163.

Tosi, E.A., Ré, E., Lucero, H., Masciarelli, R. 2001. Dietary fiber obtained from amaranth (*Amaranthus cruentus*) grain by differential milling. *Food Chem.* **73**: 441-443.

Tucker, J.B. 1986. Amaranth: the once and the future crop. *Biosci*. **36**: 9-13.

Turtola, S., Manninen, A.M., Rikala, R., Kainulainen, P. 2003. Drought stress alters the concentration of wood terpenoids in Scots pine and Norway spruce seedlings. *J. Chem. Ecol*. **29**(9): 1981-1995.

Unlu, N.Z., Bohn, T., Francis, D., Clinton, S.K., Schwartz, S.J. 2007. Carotenoid absorption in humans consuming tomato sauces obtained from tangerine or high β carotene varieties of tomatoes. *J. Agric. Food Chem.* **55**(4): 1597-1603.

University of the District of Columbia. "Peppers" (PDF). Center for Nutrition, Diet and Health. www.udc.edu.com. Retrieved 13/03/2013.

University of Illinois Extension. Lettuce". Retrieved 25/03/2013.

USDA, NRCS. 2015. The PLANTS Database. National Plant Data Team, Greensboro, NC 27401-4901 USA. (http://plants.usda.gov, 20/03/2015).

USDA nutrient database.

Vickers, C.E., Gershenzon, J., Lerdau, M.T., Loreto, F. 2009. A Unified Mechanism of Action for Volatile Isoprenoids in Plant Abiotic Stress. *Nat. Chem*. *Bio*. **5**: 283-291

van Poppel, G., Goldbohm, R.A. 1995. Epidemiologie evidence for beta-carotene and cancer prevention. *Am. J. Clin. Nutr.* **62**: 1393S-14402S.

Waller, G.R. 1987. Allelochemicals: role in agriculture and forestry. ACS Sym Ser. Pp 330.

Walling, L.L. 2000. The Myriad Plant Responses to Herbicide. *J. Plant Growth Reg*. **19**: 195-216.

Wang, W., Vinocur, B., Altman, A. 2003. Plant responses to drought, salinity and extreme temperatures: towards genetic engineering for stress tolerance. *Planta*. **218**: 1-14.

Wang, W., Vinocur, B., Shoseyov, O., Altman, A. 2004. Role of Plant Heat-Shock Proteins and Molecular Chaperones in the Abiotic Stress Response. *Trends Plant Sci*.. **9**: 244-252.

Watts, D. 2007. *Dictionary of Plant Lore*. Academic Press. Pp 226.

Webster, T.M. 2006. Weed survey- southern states. Vegetable, fruit and nut crops subsection. *Proc*. *Southern Weed Sci*. *Soc*. **59**: 260-277.

Weston, L.A. 2005. History and Current trends in the use of allelopathy for weed management. *Hort*. *Tech*. **15**(3): 529-534.

Wu, H., Patley, J., Lemerle, D., Haig, T., An, M. 2001. Screening Methods for the Evaluation of Crop Allelopathic Potential. *Bot*. *Rev*. **67**: 403-415.

Yaacob, J.S., Taha, R.M., Mat Nor, N.A., Aziz, N. 2004. Pigment analysis and tissue culture of *Amaranthus cruentus* L. First International Symposium on Sustainable Vegetable Production in South East Asia. Eds.: De Neve, S., Boehme, M., Everaarts, A., Neeteson, *J. Acta*. *Hort*. 958, ISHS 2012.

Yangzhou, J.Q., Stuttgart, M.K. 1999. Characterization of *Amaranthus cruentus* and *Chenopodium quinoa* Starch. Research Papers. Starch/Stärke. **51**(4):116-120.

Zhang, Y. 2013. Ascorbic Acid in Plants. *Biosynthesis, Regulation and Enhancement.* SpringerBriefs in Plant Science. ISBM: 978-1-4616-4126-7.

Zingaretti, S.M., Inácio, M.C., Pereira, L.M., Paz, T.A., de Castro França, S. 2013. Chapter 7: Water Stress and Agriculture. INTECH open access. http://dx.doi.org/10.5772/53877. Pp 151- 179.

CHAPTER 3

MATERIALS AND RATIONALE

3.1 Materials

3.1.1 Plant material

Plants were grown at the Faculty of Agriculture, University of the Free State, 2014-2015 in the Climate controlled cabinets (Controlled Environment LTD, Winnipeg Manitoba Canada). 29°07'S; 26°11'N.

Amaranthus cruentus (Cv Anna) seeds (harvested from Potchefstroom and Taung) were planted in pots containing a soil-compost (80:22 v/v) mixture which was placed in plastic bag lining pots with a capacity of three kilograms. Once seeds were planted, the soil was watered to field capacity; the plastic bags were sealed till seeds emerged after 3-5 days. After plants emerged the plastic bags were opened. Pots were placed in three different climate controlled chambers at 28/21°C; day/night temperatures (optimal growth temperatures) till maturity at three months. Amaranth plants grow rapidly and can be harvested 30-55 days after being planted (DAFF, 2010). The harvesting time for amaranth is not set in stone therefore a pilot study was carried out to determine when the best time would be to harvest (DAFF, 2010). This was done to see that there was enough plant material for all experiments. There were 50 pots per chamber with an average of three plants per pot. Daylight was 12 hours in order to prevent flowering. Plants were watered every second day. Ten weeks after germination plants were fed with hydroponic solution (Chemicult), left for another 2 weeks, thereafter stress treatment commenced.

Vegetable seeds used in this study were obtained from Starke Ayres: Money Maker Tomato, California Wonder Sweet Pepper, Ashley Cucumber and Great Lakes Lettuce.

3.1.2 Other materials

All the chemicals used, i.e. methanol, dichloromethane (DCM), acetic acid, aluminium chloride, sodium carbonate, sodium hydroxide and agar were of the purest grade available and purchased from Merck (Germany). Aluminium thin-layer chromatography plates (Silica gel 60 F254; 20 x 20 cm^2) were also purchased from Merck (Germany). Folin-Ciocalteau Reagent and Sephadex® LH-20 were purchased from Sigma-Aldrich (Germany).

3.2 Methods

3.2.1 Temperature stress treatment

Three month old plants were subjected to cold (14/7°C) and hot (40/33°C) stress temperatures, while other plants were left at optimal (28/21°C) day/night temperatures where no stress was experienced, with a 12 hour light period. The plants were grown at these temperatures for 14 days; water was kept at field capacity throughout the stress period. All temperature surviving plants were harvested after 14 days. The aerial parts of the plants were placed in plastic bags, transported in a cooler with ice and stored at -70 °C until further analysis. Optimal temperature conditions serve as the control.

3.2.2 Preparation of crude plant extracts

Leaf samples were ground in liquid nitrogen and 10 g successively extracted using methanol-water (70:30) and dichloromethane as solvents (1:20 w/v). The pooled extract was dried using a rotavapor (Büchi rotavapor R-3 with a vacuum pump V-700, Germany) at 40°C. The moist aqueous solution was further dried for 2-5 days, in a freeze dryer (Freezemobile ll, Virtis Company Inc.), removing the left over water. The mass of the dried extract was determined.

3.2.3 Total phenolic concentration

The total phenolic content was determined by using the Folin-Ciocalteu assay (Singleton & Rossi, 1965). A methanol extraction of the samples (1:10 w/v) was prepared through shaking the samples for 16 hours at 1660 rpm (Heidolph Multi Reax, Labtec shaker). Gallic acid was used as standard solution (100, 200, 300, 400, and 500 µg mL^{-1}). An aliquot (1 mL) of extracts or standard solution of gallic acid was added to a 25 mL volumetric flask, containing 9 mL of distilled water. One millilitre of Folin-Ciocalteu phenol reagent was added to the mixture and shaken. After 5 minutes 10 mL of 7% Na_2CO_3 solution was added to the mixture. The volume was then made up to the mark. A reagent blank using only distilled water was prepared exactly the same. After incubation for 90 minutes at room temperature, the absorbance against the reagent blank was determined at 550 nm with an UV-Visible

spectrophotometer (BioTek EKx808). Total phenolic content was expressed as mg gallic acid equivalents (GAE). All the assays were performed in triplicate.

3.2.4 Total flavonoids

Total flavonoid content was measured by the aluminium chloride colorimetric assay (Zhishen *et al*., 1999). Methanol extractions of the samples (1:10 w/v) were prepared through shaking the samples for 16 hours at 1660 rpm (Heidolph Multi Reax, Labtec shaker). Quercitin was used as standard solution (20, 40, 60, 80 and 100 µg mL^{-1}). An aliquot (1mL) of extracts or standard solutions of quercetin was added to a 10 mL volumetric flask containing 4 mL of distilled water. To the flask was added 0.30 mL 5% $NaNO_2$ and after five minutes 0.3 mL 10 % $AlCl_3$ was added. After another five minutes, 2 mL 1M NaOH was added and the volume was made up to 10 mL with distilled water. A blank was prepared in the same manner by using distilled water. The solution was mixed and absorbance was measured against the blank at 510 nm (BioTek EKx808). The total flavonoid content was expressed as mg quercetin equivalents (QE). All the assays were performed in triplicate.

3.2.5 Chromatographic techniques

3.2.5.1 Thin Layer Chromatography

Thin layer chromatography (TLC) was carried out using silica gel 60 F_{450}–aluminium backed pre-coated plates. Extracts were dissolved in their appropriate extraction solvents at 50 mg mL^{-1} and 10 µL applied to the TLC. The mobile phase for development of the aqueous methanol extracts was chloroform-methanol-water-acetic acid (65:35:5:1), while for the DCM extracts, the mobile phase in which the plates were developed comprised of toluene-ethyl acetate (93:7). Compounds resolved on the plate were visualized using either general or specific methods. Ultraviolet light (UV) indicates fluorescent compounds. They were examined at 365 nm (long) and at 254 nm (short) wavelength UV light (Vogelmann & Evans, 2002). Alternatively, colourless compounds required a chemical reaction in order to visualize their location (Smith & Seakins, 1976). Spray reagents to produce coloured derivatives were used, namely ninhydrin (Pifrung, 2006), *p*-anisaldehyde-sulphuric/acetic acid, 5% ferric chloride and dragendorf reagents, prepared according to the standard methods described by Wagner & Bladt (1996).

3.2.5.1.1 Antioxidant and antibacterial activity

A 0.2% 2,2-diphenyl-1-picrylhydrazyl (DPPH) reagent was used to illustrate compounds with antioxidant activity on TLC plates (Glavind & Holmer,1967).

A direct bioautographic assay on TLC for compounds with activity against gram-positive and gram-negative bacteria were used as described by Hamburger *et al*. (1987). Two mL suspensions of *Bacillus subtilis* (gram-positive) and *Escherichia coli* (gram-negative) in nutrient broth were applied to developed TLC plates (15 x 10 cm). Plates were placed into containers that were sealed to keep moisture in and incubated at 35 °C overnight. Incubation in a humid atmosphere permits growth of the bacteria. Plates were then sprayed with a 2 mg mL^{-1} dehydrogenase-activity-detecting reagent, i.e., *p*-iodonitrotetrazolium violet salt (INT) and incubated for another 24 h. Metabolically active bacteria convert the tetrazolium salt into the corresponding intensely coloured formazan (purple) background and zones of inhibition were visualized as white spots.

3.2.5.2 High pressure liquid chromatography

The methanol-water extracts of the different treatments were analysed by high pressure liquid chromatography (HPLC) on a Shimadzu instrument (Tokyo, Japan; CBM 20A-communication bus module, DGU 20AS-degasser, LC 20AD-pump A & B, CTO 10AS UP-column oven) with a Photo Diode Array Detector (PDA). The elution procedure to achieve an acceptable separation of all compounds was described by Vidović *et al*, (2015).

A reverse phase column (Phenomenex C_{18}, 250mm × 4.6mm with a diameter of 5µm) with a gradient mobile phase was used. Solution A was acetonitrile and solution B contained acetic acid-acetonitrile-phosphoric acid-water (10:5:0.1:84.9, v/v/v/v). Initially 0-5 min, 100% solution B; 5-25 min, 100-80% solution B; 25-30 min, 80-60% solution B; 35-40 min, 60-100% solution B at a flow rate of 1 mL min^{-1} and constant temperature of 25°C. The eluate was detected by measuring the absorbance between 200 and 400 nm.

3.2.5.3 Gas chromatography and mass spectrometry

Mass spectrometry of compounds and reaction mixtures were performed by means of electron impact (EI) ionization making use of a Shimadzu GC-MS QP-2010 gas chromatograph-mass spectrometer fitted with a DB-5 MS column (30 m, 0.32 mm i.d., 0.25 µm film thickness). Carrier gas was helium at a flow rate of 1.5 mL min^{-1}. Column temperature was initially kept at 60°C for 5 min, then gradually increased to 280°C at a rate of 2°C min^{-1}, and held for 10 min. Dichloromethane samples (1 µL, appropriately diluted in hexane) were injected at 280°C with split mode (1:50). The concentration of the samples was 10 mg mL^{-1}. Flame ionization detector (FID) was set at 280°C. Mass spectrometer was used to identify components separated by GC according to their retention indices and GC-MS fragment patterns. The identification of components was done with the use of the National Institute of Standard and Technology (NIST) version 5.0 library.

3.2.6 Allelopathy

The 'sandwich method' of Fujii *et al*. (2003) was used for determining the *in vitro* phytotoxicity of the leaf litter and extracts from the different treatments of *A. cruentus* on various vegetable seeds. A 5 mL layer of 0.5% (w/v) sterile water agar was poured into each well of the sterile multi-dish plate (Fig 3.1) and allowed to set. The *A. cruentus* leaf litter or extract (methanol-water or DCM) was placed on this bottom layer and a second layer of 5 mL sterile water agar was added on top. This made a sandwich of dried leaves or extracts by the two layers of agar (10 mL in total). This method was developed in order to physically separate the *A. cruentus* samples from the seed, however allows for diffusion of any active component from the sample through the barrier agar layer.

Vegetable seeds were surface sterilised by washing in 96% ethanol for 1 minute followed by 1.30 min in 3,5% NaCl and finally back into ethanol for 30 seconds. After sterilization the seeds were placed on sterile filter paper and left to dry within a laminar flow cabinet (Labotec, airflow from top). The seeds were then positioned vertically on the top layer of the agar and incubated at 25°C. Germination and growth were monitored.

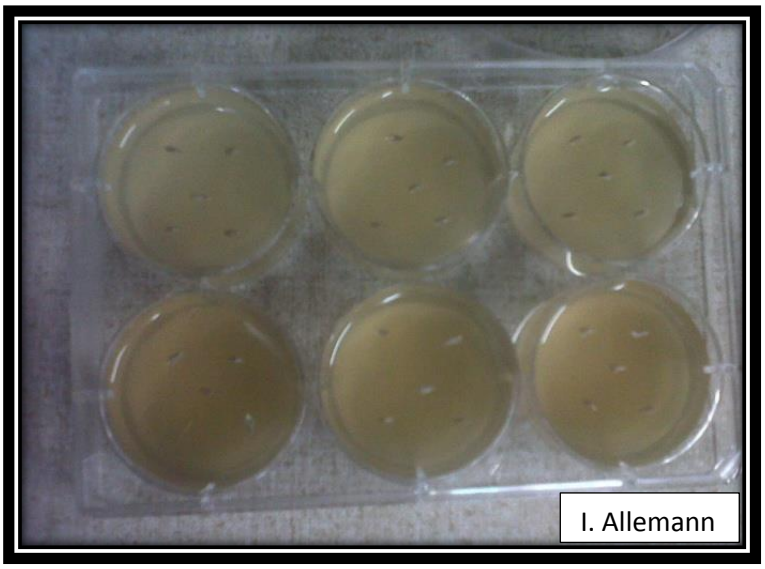


Figure 3.1: Six-well dish with agar used in Sandwich method bioassay.

3.2.6.1 Leaf litter

The leaves of the different temperature stressed plants were harvested, stored at -70°C and lyophilised, then ground into a fine powder using a grinder. The phytotoxicity of 10 and 50 mg of the powdered leaf material was used in the sandwich method (1 and 5 mg mL^{-1} litter per well) against lettuce, tomato, cucumber and sweet pepper seeds (Fujii *et al.,* 2003). Controls contain no leaf material.

3.2.6.2 Extracts

Combining the sandwich method and a modified method from Hill *et al.* (2007), filter paper was used to retain the plant extracts between the agar layers. Five and twenty mg of each extract was dissolved in 1 mL of their own solvent, DCM or methanol-water and pipetted onto a filter paper (Whatman No.40 11 cm, Ashless), allowed to dry in a fume hood then placed on the bottom layer of agar resulting in 0.5 or 2 mg mL^{-1} extract per well. Controls contain only the solvents on filter paper (Fig 3.2).

3.2.6.3 Growth conditions and growth measurement

After placing the seeds on the top layer of agar in each well, plates were closed and incubated at 25°C in black bags (full darkness) for 3-7 days depending on the vegetable used. On the 3^{rd} to 7^{th} day the germination percentage, length of the

radicle and hypocotyl was measured. Each of the experiments was done in triplicate and presented as the mean of the replicates.

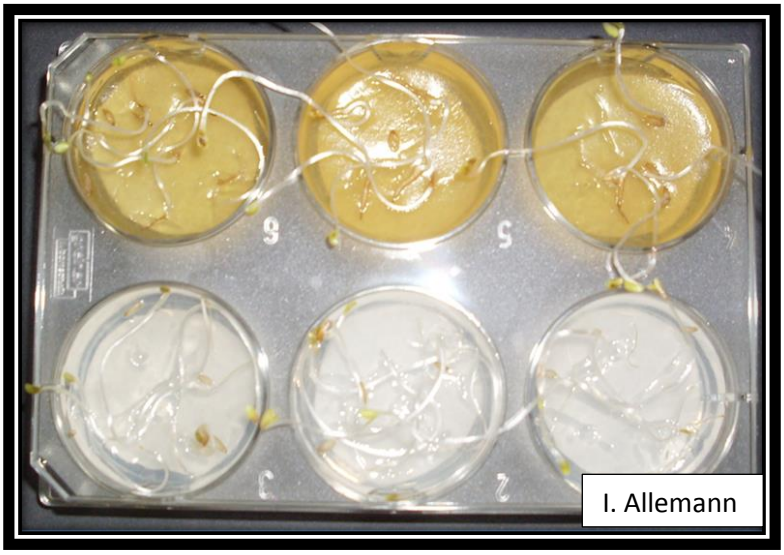


Figure 3.2: Lettuce seeds incubated on filter paper with *A. cruentus* extract in the top row and on control filter paper in the bottom row.

3.2.7 Statistical analysis

The results were expressed as means with least significant difference (LSD). Analysis of variance (ANOVA) was performed using SAS 9.3 (Institute Inc., Cary, NC, USA, 2008) statistical programme for data and Tukey-Kramer's LSD procedure for comparison of means. Significance of differences compared to the control groups was determined using the t-test (Steel & Torrie, 1980).

References

Department of Agriculture, Forestry and Fisheries. 2010. Amaranthus: Production guideline. *DAFF.* Pp 1-14.

Fujii, Y., Parvez, S.S., Parvez, M.M., Ohmae, Y., Iida, O. 2003. Screening of 239 medical plant species for allelopathic activity using the sandwich method. *Weed Bio*. *Man*. **3**: 233-241.

Glavind, J., Holmer, G. 1967. Thin-layer chromatographic determination of antioxidants by the stable free radical α, α'- diphenyl-β-picrylhydrazyl. *J*. *Am*. *Oil Chemists' Soc*. **44**(9): 539-542.

Hill, E.C., Ngouajio,M., Nair, MG. 2007. Allelopathic potential of Hairt Vetch (*Vicia villosa*) and Cowpea (*Vigna unguiculata*) Methanol and Ethyl Acetate Extracts on Weeds and Vegetables. *Weed Tech*. **21**: 437-444.

Hamburger, M.O., Cordell, G.A. 1987. A direct bioautographic TLC assay for compounds possessing antibacterial activity. *J*. *Nat*. *Prod*. **50**(1): 19-22.

Pifrung, M.C. 2006. The Synthetic Chemists Companion. Wiley. Online Library. http//www.onlinelibrary.wiley.com/book/10.1002/9780470141045 (15/09/2013).

Singleton, V. L., Rossi, Jr J.A. 1965. Colorimetry of total phenolics with phosphomolybdic phosphotungstic acid reagents*. Am*. *J*. *Enolo. Viticul*. **16**: 144-158.

Smith, I., Seakins, J.W.T. 1976. Chromatographic and electrophoretic techniques. London: William Heinermann Medical Book Ltd. Pp 158-159.

Steel, R. G. D., Torrie, J. H. 1980. Principles and Procedures of Statistics, Second Edition, New York: McGraw-Hill. Pp 633.

Vidović, M., Morina, F., Milić, S., Jovanović, S.V. 2015. An improved HPLC-DAD method for simultaneously measuring phenolics in the leaves of *Tilia platyphyllos* and *Ailanthus altissima*. Institute of Botany and Botanical Garden Jevremovac, Belgrade. *Botanica SERBICA*. **39** (2): 177-186.

Vogelmann, T.C., Evans, J.R. 2002. Profiles of light absorption and chlorophyll within spinach leaves from chlorophyll fluorescence. *Plant, Cell Environ*. **25**(10): 1313-1323.

Wagner, H., Bladt, S. 1996. Plant drug analysis. A thin layer chromatography atlas. Springer-Verlag, Berlin, Heidelberg. Pp 1-384.

Zhishen. J., Mengcheng. T., Jianming. W. 1999. The determination of flavonoid contents in mulberry and their scavenging effects on Superoxide radicals, *Food Chem.* **64**: 555-559.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Pant survival and yield after temperature stress treatment

The environment can be defined as the collective of all external conditions which have an influence on growth and development of plants (Rajasekar *et al*., 2013). Light, temperature and relative humidity are among the environmental factors which play an important role in crop growth and development. High or low temperatures affect seed germination and seedling establishment in a field (Horak & Loughin, 2000; Svirskis, 2003). Since amaranth is becoming an alternative crop in different parts of the world it is essential to understand the importance of temperature on the survival of the plant.

The results of different temperatures on the survival of *A. cruentus* plants can be seen in Table 4.1, where it is clear that cold stress (14/7°C) is the most detrimental with a 72% survival rate. Optimal (28/21°C) and hot temperatures (40/33°C) had no effect on the survival rate of the plants. *Amaranthus* species are C4-plants and it is well-known that C4- plants are generally tropical or subtropical, therefore are more resistant to heat (Hopkins & Hüner, 2009). Photorespiration is very low or absent in C4-plants while photosynthesis remains constant with increasing leaf temperatures (Hopkins & Hüner, 2009). Most C4-plants perform poorly or die when exposed to colder temperatures as can be seen in Table 4.1. In maize, the plant stops growing below 12° to 15°C. This limited growth is most likely caused by the enzyme pyruvate, phosphate dikinase, which is cold sensitive and in colder temperatures experiences a large loss of activity. When this enzyme stops working it inhibits the regeneration of phosphoenol pyruvate (PEP), which is an important substrate for PEP carboxylase, the key enzyme in C4 photosynthesis (Hopkins & Hüner, 2009).

Table 4.1: Total and percentage surviving *A. cruentus* plants at cold (14/7°C), optimum (28/21°C) and hot (40/33°C) temperatures.

Treatment	Total surviving plants / 150	Survival rate (%)
Cold	147	98
Optimal	150	100
Hot	108	72

Exposure to temperatures, at higher (40/33°C) or lower (14/7°C) than optimal, resulted in only slight changes in total fresh mass of plants compared to those plants grown at optimal temperatures (28/21°C) (Table 4.2). The total average fresh mass of plants grown at lower temperatures was only 1.32% greater than that of plants grown at the optimal temperature, while at high temperatures (40/33°C) the average fresh mass of plants decreased by 8.2%. However, considering that only 72% of the cold stressed plants survived, the fresh and dry masses per plant were significantly higher than the other treatments (Table 4.2).

Table 4.2: Influence of temperature on total fresh and dry leaf yield, leaf yield per plant as well as percentage water in fresh leaves.

Treatment	Total leaf mass (g)		Leaf mass per plant (g)		Water (%)
	Fresh	Dry	Fresh	Dry	
Cold	1093.8	190.8	10.13	1.77	82.56
Optimal	1079.5	154.2	7.34	1.05	85.72
Hot	991.2	81.3	6.61	0.54	91.79

The fresh mass per plant grown at cold temperatures, was 35% and 28% more than the hot and optimal treatments respectively, while the dry mass was 70% and 41% more than the hot and optimal treatments. It was found that plants at the lower temperatures ranges exhibited thicker, slightly curled leaves compared to those grown at both optimal and hot temperatures. This could be because the plants were protecting themselves from freezing (Wang, 1982) and contributed to the increase in leaf mass.

These findings are contradictory compared with research conducted by Guo & Al-Khatib (2003) on other amaranth species namely, redroot pigweed, Palmer amaranth and common water hemp, where all three species produced less biomass at colder temperatures than at warmer temperatures. Guo & Al-Khatib (2003), found that *Amaranthus* species were stunned and the plants overall growth was slower at colder temperatures. These conflicting results may be as the result of the difference in cold stress treatment. In our study, plants were cold stressed after grown at

optimal temperatures while in these author's case, plants were grown from seedling stage at the colder temperatures.

Initiation and expansion of roots, leaves, shoots, tillers, branches and reproductive organs are strongly driven by temperature. Warmer conditions both accelerate rate of organ initiation and shorten duration of organ growth. In cooler temperatures leaves are often larger than in warm temperatures because of a slow rate of expansion but long duration (Morrison & Lawlor, 1999).

Total fresh and dry weights of the leaves were noted and the loss of water was calculated for each of the stress treatments (Table 4.2). After plants were lyophilised and weighed it showed that the water content of the plants exposed to cold temperatures was the lowest at 82.56%, which correlates with the 86% moisture content that was found in a study by Mensah *et al.* (2008). Heat treated plants had the highest moisture content (91.79%) of the treatments. Plant species that grow in arid regions (hotter temperatures, low rainfall) have physical changes including having thicker cuticles than plants that grow in moist habitats. This thicker cuticle reduces water loss though transpiration (Stern, 2006). C4-plants are able to close their stomata temporarily when the temperature is too high and this happens without limiting photosynthesis.

4.2 Extraction of plant material

Production of secondary metabolites occurs when plants are under stress (Bertin *et al*., 2003), therefore understanding the significance of temperature stress on the production of these metabolites will shed light on the allelopatic effect of amaranth and determine what the long term impact will be on succeeding crops in crop rotation or intercropping practises.

Studies by Kothari & Seshadri (2010) on seed extracts showed that a higher yield extraction was directly proportional to anti-bacterial activity of higher magnitude. The extraction process can thus be regarded as a crucial step in any plant formulation before running bioassays. Many solvents are used to extract phytochemical compounds from plant material. An important factor governing the choice of solvents used in an extraction is the type of phytochemical groups that are targeted for extraction if they are known (Houghton & Raman, 1998). In this study, no particular

phytochemical group was targeted for extraction as it was not established which compounds could have alleopathic activity. The solvents chosen for extraction purposes were dichloromethane and methanol-water (70:30), which had varying polarities with different chemical properties and allow for the extraction of different compounds from the plant material used. The different solvents therefore provide a broad spectrum of possible phytochemicals that can be extracted from the different treatments of *A. cruentus* leaves. The mass that each solvent extracted from 1 g dried leaf material was determined, calculated as percentage (g/g) extracted and recorded in Table 4.3.

Table 4.3: Plant extracts recovery obtained by different solvents.

	Percentage mass litter extracted		
Treatment	**Solvent used for extraction**		
	Methanol-water	**DCM**	**Average %**
Cold	14.03	3.57	**8.8**
Optimal	9.9	3.5	**6.7**
Hot	14.87	5.5	**10.19**
Average %	**12.93**	**4.19**	

Methanol-water is a polar extractant and has the ability to extract numerous compounds including terpenoids, phenolics, sugars, amino acids, anthocyanins, saponins and polypeptides (Houghton & Raman, 1998; Bart, 2011). The high percentage mass recovery resulting from the extraction with methanol-water was primarily due to the fact that compounds present in the *A. cruentus* leaves being hydrophilic in nature. Dichloromethane is a non-polar solvent that can extract compounds such as non-polar phenolics, terpenoids and alkaloids (Guillen & Manzanos, 1998). A pilot study done by Allemann (2013), revealed the presence of steroids, saponins, phenols, tannins, terpenoids and glycosides in extracts from the amaranth cultivar Anna.

4.3 Total phenolic and flavonoid concentration

The total phenolic (TPC) and flavonoid contents of the leaves of three temperature treated, cold, hot and optimal (stress control), *A. cruentus* plants were expressed as mg gallic acid (GAE) and mg quercetin equivalent (QE) per gram of dry weight (D.W.) respectively. Results indicate that *A. cruentus* plants exposed to optimal temperatures, significantly contained the highest concentration of phenolic compounds followed by cold and lastly hot conditions (Figure 4.4). Total flavonoid content followed the same trend. These results are in contrast with findings of many authors who reported an increase in production of phytotoxic phenolic compounds in plant tissues exposed to high temperatures and solar radiation (Koeppe *et al*., 1969; Wender, 1970; Einhelig & Eckrich, 1984). Wang & Zheng (2001), cultivated strawberry plants under different combinations of night and day temperatures (12/18 °C, 12/25 °C, 22/25 °C, 22/30 °C) and examined the composition of phenolic compounds, as well as the antioxidant capacity of the juice. The highest night and day temperatures resulted in the highest production of phenolic compounds. Menges (1987) said that allelopathy was enhanced as soil temperature, soil moisture, ultra-violet light or sunlight, microorganism activity and soil salinity increased.

Table 4.4: Total phenolic and flavonoid compounds in temperature stressed amaranth leaf material.

Treatment	Total phenolic content (mg GAE/g D.W.)	Total flavonoid content (mg QE/g D.W.)
Cold	12.0 b	5.4 a
Optimal	18.8 a	5.6 a
Hot	10.1 b	4.1 b

In plants heat-shock responses occurred when living cells are exposed to temperatures that are well above their normal growth temperatures (Loaiza-Velarde *et al*., 1997). In our study plants were grown at optimal temperatures for 3 months, where after temperature stress was introduced, therefore, the plants experienced a temperature shock. The decrease in phenolic composition maybe as a result of the induction of heat-shock proteins accompanied by a decrease in the enzyme

phenylalanine ammonia-lyase (PAL), a key enzyme in the phenylpropanoid metabolism, however follow-up studies have to be done to measure PAL activity. A study done by Loaiza-Velarde *et al*. (1997) on lettuce showed an initial increase in PAL activity after wounding, but after plants were exposed to heat treatment of 45, 50 and 55°C, the PAL activity and concentration of phenolic compounds decreased .

In peas grown at optimum and low temperatures, total phenol content in the two treatments differed insignificantly, however, low growth temperature decreased the content of some phenolic compounds in pea seedling roots (Rudikovskaya *et al*., 2008). Long-term drought stress caused a decrease in selected components of secondary metabolism in the leaves and roots of grapevine (Król *et al*., 2014). It seems therefore that one cannot expect generalized patterns of phenolic compounds in stress situations.

Various phenolic compounds and flavonoids were reported previously in amaranth seeds and vegetables. For instance, the sprouts of amaranth contained rutin as the main constituent and gallic, p-coumaric and syringic acids as other important constituents (Paśko *et al*., 2008). Kalinova & Dadakova (2009) referred to the varying concentrations of rutin in different Amaranth species; however *A. hybridus* and *A. cruentus* were rich sources of rutin. In a study done by Steffenson *et al*. (2011), it was found that the content of phenolics in *Amaranthus* seeds is generally quite easily influenced by environmental factors and that rutin, exhibited large variations with varying environmental conditions.

4.4 Chromatography

4.4.1 Thin layer chromatography

In order to determine and visualise the location of various compounds, thin layer chromatography (TLC) studies were carried out as a primary screening process. Thin layer chromatography provides a quick, cost effective way in the identification of compounds in plants. The compounds that were separated on the TLC plates were identified based on their R_f values, developing system that was used for the separation and the colour reaction of the compounds when detection spray reagents were applied. Figures 4.1 and 4.2 illustrate the TLC results for MeOH-H_2O (polar) and DCM (non-polar) leaf extracts of the different temperature treatments of *A.*

cruentus plants. Different compounds with varying R_f values were visible under UV light and with detection spray reagents.

Under UV-256 nm light, blue spots with different R_f values were visible in both the polar and non-polar extracts. Fewer spots at lower intensities were visible in both extracts exposed to the high temperature treatment (Fig 4.1a & 4.2a). According to Wagner & Bladt (1996), under UV light the blue fluorescent spots indicate compounds with conjugated double bonds. These compounds may represent alkaloids, flavonoids or triterpenes (Wagner & Bladt, 1996). In the DCM extracts, light to dark green spots (R_f = 0.08, 0.09, 0.13, 0.16) were visible in all three treatments under this illumination and could be chlorophyll pigments. On the baseline, there were very polar compounds that were unable to migrate up the plate (Fig. 4.2a & b). In both the polar and non-polar extracts, under 365 nm, there were no visible fluorescent compounds for any of the treatments.

Ferric chloride ($FeCl_3$) solution was used to determine the presence of phenolic compounds and the blue-grey colour spots represented compounds with a phenol ring (Jork *et al.,* 1990). The use of ferric chloride with heating shows the colour change and in visible light the grey spot on the plate is visible due to the d-d transitions in the octahedral complex between the Fe^{3+} ion and the hydroxyl groups of phenols (Rungsimakan, 2011). All the polar samples contained phenolic compounds, although the heat treated plant extracts had less spots than the other treatments (Fig 4.1b). The optimal and cold treatment polar extracts showed prominent blue-grey spots at R_f = 0.05, 0.48, 0.89, and 0.92, while in the heat treatment there was only one spot at R_f = 0.05, indicating the presence of phenolic compounds (Fig 4.1b). In the non-polar extracts (Fig. 4.2b) grey spots were not as clear, although on the bottom of the plate spots were visible. Phenolic compounds have been shown to play a role in allelopathic interactions among different groups of plants such as bryophytes, pteridophytes, gymnosperms, and angiosperms (Rice, 1979; Fisher, 1987; Inderjit, 1994; Lawrey, 1995).

After spraying the TLC's with the general detection reagent *p*-anisaldehyde-sulphuric/acetic acid, many different compounds could be observed (Fig. 4.1c & 4.2c). There was a wide range of colours from green, yellow, pink, blue and purple with different R_f values (Table 4.5 & 4.6) for the polar and non-polar extracts. These

may indicate many different compounds, including terpenes, saponins, sugars and flavonoids amongst others (Wagner & Bladt, 1996). Kraujalis *et al*. (2013) reported on the antioxidant properties and phytochemical composition of amaranth extracts isolated by acetone and methanol-water from plant leaves, flowers, stems and seeds. They found that the methanol-water extract of the leaves possessed the highest antioxidant activities and various phenolic compounds and flavonoids e.g. rutin, nicotiflorin, isoquercitrin, 4-hydroxybenzoic and *p*-coumaric acids were identified as major constituents. In the review article by Mroczek (2015) it is reported that saponins were isolated from a diversity of Amaranthaceae genera and species.

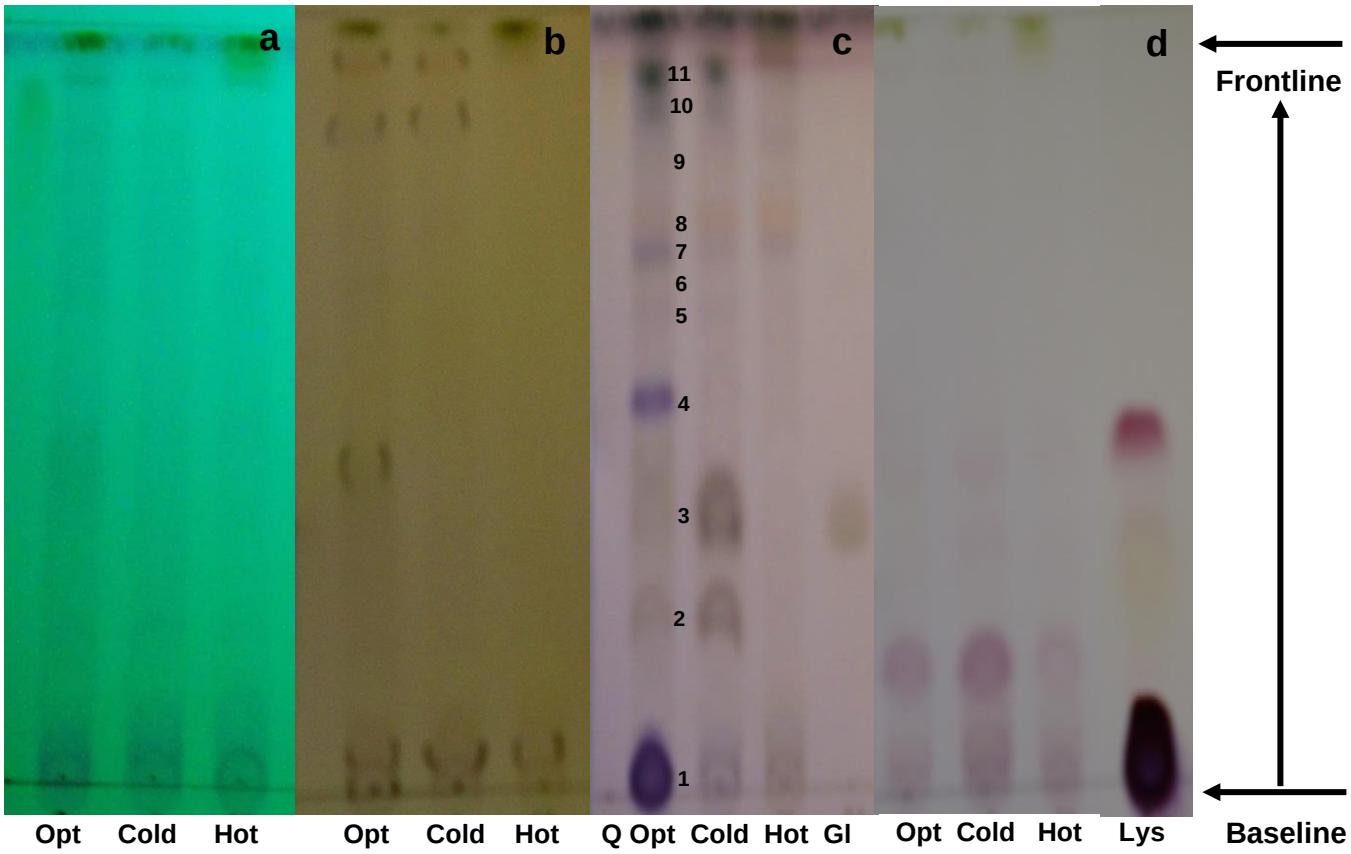


Figure 4.1: Qualitative TLC profiles of the optimal (Opt), cold and hot treated *A. cruentus* polar water-methanol leaf extracts. Standards: Q = quercetin; Gl = glucose and Lys = lysine. Different detection methods were used to visualise compounds: a = UV 254 nm; b = $FeCl_3$; c = *p*-anisaldehyde; d = ninhydrin. Plates were developed in $CHCl_3$-MeOH-H_2O-acetic acid [65:35:5:1%]

Table 4.5: Colour and R_f values of compounds in the methanol-water leaf extracts of the different temperature treated *A.cruentus* plants, visualised by *p*-anisaldehyde spray reagent on TLC.

Compound	R_f-value	Colour of compound		
		Optimal	**Cold**	**Hot**
1	0.055	Purple		
2	0.24	Green-Brown	Green-Brown	
3	0.35	Grey	Grey	Grey
4	0.49	Blue-Purple		
5	0.6	Light blue		
6	0.64	Yellow	Yellow	Yellow
7	0.69	Dark blue	Dark blue	Dark blue
8	0.7	Pink	Pink	Pink
9	0.72	Yellow	Yellow	Yellow
10	0.86	Blue-Green	Blue-Green	
11	0.9	Green	Green	
Quercitin	0.88	Yellow		
Glucose	0.35	Grey		

From our study, it is clear that temperature played an obvious role in the production of secondary compounds as clear differences in compounds between the treatments were visible in both the polar and non-polar extracts (Fig. 4.1c & 4.2c). In the optimal treatment of the polar extract, 11 compounds were noted, compared to 8 and 5 in the cold and hot treated samples respectively. Prominent spots, including a dark purple (R_f = 0.055), a blue-purple (R_f = 0.49) and a light blue spot (R_f = 0.6) were only present in the optimal extract.

From these results one can deduct that the stress temperatures, particularly the hot, inhibited the biosynthesis of some of the more polar compounds. Differences were also visible in the non-polar samples (Fig. 4.2c), with a noticeable blue coloured compound visible at R_f = 0.83, solely in the hot treatment DCM extract. Less green

pigment, probably chlorophyll was also observed in the hot treatment extract, indicating the effect the hot treatment had on photosynthesis. Several studies have examined the effects of increased temperatures on secondary metabolite production of plants (Morrison & Lawlor, 1999). Lower soil temperatures caused an increase in levels of steroidal furostanol and spirostanol saponins (Szakiel *et al*., 2010).

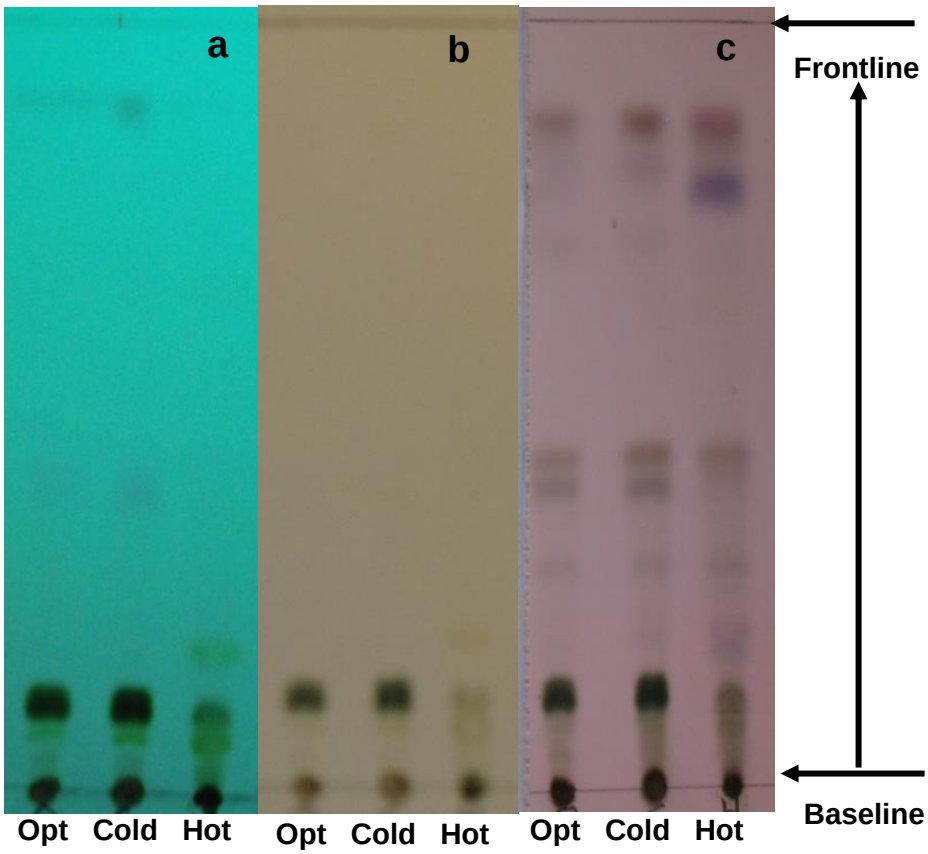


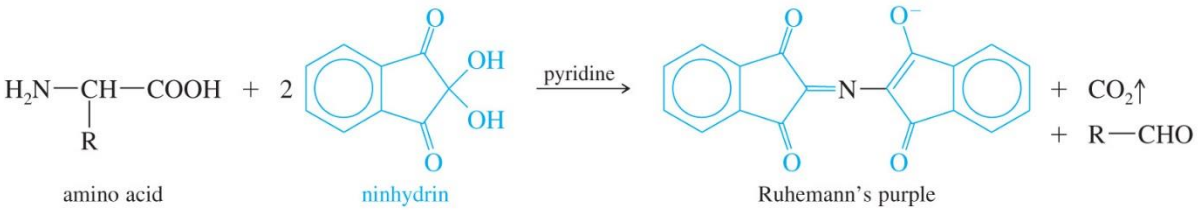
Figure 4.2: Qualitative TLC profiles of the optimal, cold and hot treated *A. cruentus* non-polar DCM leaf extracts. Different detection methods were used to visualise compounds: A = UV 254 nm; B = $FeCl_3$; C = *p*-anisaldehyde. Plates were developed in Toluene-ethyl acetate [93:7].

Table 4.6: Colour and R_f values of compounds in the DCM leaf extracts of the different temperature treated *A.cruentus* plants, visualised by *p*-anisaldehyde spray reagent on TLC.

Distance	Rf-value	Colour of pigment		
		Optimal	**Cold**	**Hot**
1	0.13	Dark-Green	Dark-Green	Light-Green
2	0.19		Purple-Blue	Purple
3	0.21		Purple-Pink	Purple-Pink
4	0.29	Grey	Grey	Dark-Grey
5	0.39	Green-Grey	Green-Grey	
6	0.43	Green-Brown	Green-Brown	Green-Brown
7	0.83			Blue
8	0.9	Pink	Pink	Pink

Amino acids were visualised by spraying the developed TLC plates with ninhydrin solution. The reaction of amino acids with ninhydrin results in the formation of purple coloured complexes as shown in the reaction below.

Reaction with ninhydrin:



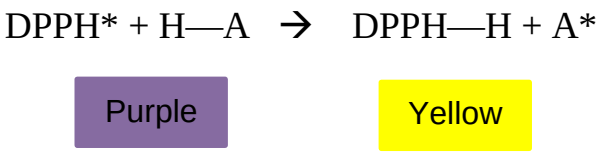
Amino acids were restricted to the polar extracts (Fig 4.1d). Lysine (R_f = 0.16, 0.69) was present in all three treatments when compared to the standard. The composition of amino acids in amaranths was reported to be close to animal protein with an extra ordinary high content of lysine which is 2 and 3 times higher than that in wheat and maize, respectively.

Dragendorf reagent spray was used for the detection of alkaloids, which are precursors of amino acids that may have allelopathic functioning (Wink, 1999). Bright

orange spots reveal the presence of alkaloids. No compounds were detected on the TLC plates for the presence of alkaloids (Fig. 4.1 & 4.2).

4.4.1.1 DPPH antioxidant activity

The use of DPPH reagent in a qualitative manner to determine which compounds possess antioxidant properties, proved positive for all the extracts of the three temperature treatments (Fig. 4.3a & b). DPPH measures the electron donating activity of the compound by its ability to scavenge off radicals (Lü *et al.,* 2010). The yellow spots present on the TLC plate on a mauve background represent the antioxidant compounds (Bondet *et al.,* 1997).



The compounds possessing antioxidant activity in the methanol-water extract appeared to be the more polar components of all the treatments ($R_f \leq 0.5$; Fig 4.3a) that were identified earlier as phenolic compounds (Fig. 4.1b). The heat treatment however, displayed less compounds with antioxidant activity and phenolic compounds, which were also confirmed by the reduced amount of TPC (Table 4.4). Ayoola *et al.* (2008) showed that there is a good correlation between flavonoid content and antioxidant activity.

A distinctive compound ($R_f = 0.83$; Fig. 4.3b), only present in the DCM extract of the hot treated plants displayed strong antioxidant activity. After *p*-anisaldehyde-sulphuric/acetic acid spray reagent was used (Fig 4.2b), this compound coloured blue and according to literature may be a terpene (TLC stains, 2016). Amaranth leaves are known to be high in antioxidant activity when compared to other traditional green leafy vegetables (Hunter & Fletcher 2002; Kraujalis *et al*., 2013). Antioxidant compounds protect a plant from any damage caused by oxidative stress (Gupta *et al.,* 2006).

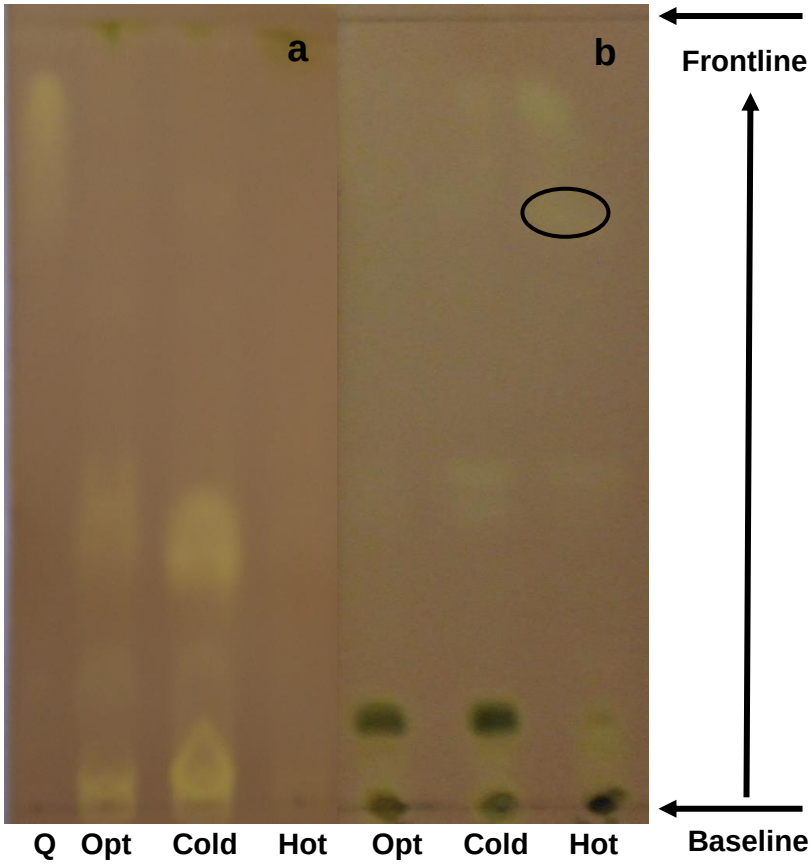


Figure 4.3: Qualitative TLC profiles of the antioxidant activity displayed by the optimal, cold and hot treated *A. cruentus* polar methanol-water **(a)** and non-polar DCM **(b)** leaf extracts. **Plate a:** developed in $CHCl_3$-MeOH-H_2O-acetic acid [65:35:5:1%] and **b:** developed in Toluene-ethyl acetate [93:7].

4.4.1.2 Antibacterial activity

No antibacterial activity against *B. subtilis* was detected; however, compounds in the hot treated methanol-water extract showed antibacterial activity against *E. coli*. The TLC plate displayed white spots indicating that there is no bacterial growth on those particular compounds (TLC not shown). These results proved that *A. cruentus* plants that were exposed to temperatures of 40/33°C produced compounds with antibacterial properties against *E. coli.* Compounds that can be responsible for this antibacterial activity can be flavonoids, terpenoids, tannins and phlobatanins which are phytochemicals found within *A. cruentus* (Maiyo *et al*., 2010). Experiments done by Broekaert *et al*., (1992) on *A. caudatus* also demonstrated their antimicrobial activity. Isoflavonoids are antimicrobial compounds produced in response to biotic

stress (Bhattacharya *et al*., 2010), therefore it is possible that antimicrobial compounds were induced upon the hot stress treatment in our study. Even though *E. coli* is a human bacterium, it may be possible that this antibacterial compound can have an influence on other soil bacteria. Therefore there will be a change in the rhizosphere in the soil which may have a possible impact on plants.

4.4.2 High pressure liquid chromatography

The polar, methanol-water extracts of the different temperature treated plant leaves were analysed by high pressure liquid chromatography (HPLC), using a method for separation of phenolic compounds as described by Vidović *et al*. (2015). Reversed-phase HPLC offers a method for obtaining metabolic fingerprints of phenolic compounds within 50 min. Separation of five standard phenolic compounds belonging to different groups e.g. phenolic acids, flavanols, flavon-3-ols and their glycosides are presented in Figure 4.4. It is clear from fig. 4.4, that the subclasses of phenolic compounds exhibit different spectral characteristics e.g. benzoic acids (gallic acid) and flavanols (catechin) absorb energy best at 280 nm, cinnamic acids and derivatives (caffeic acid) best at 320 nm and the flavon-3-ols (quercetin, rutin) best at 360 nm. When the chromatograms of the different treatments were compared at different wavelengths, variations in compounds between the treatments stood out (Fig. 4.5). The presence of catechin (Rt = 5.99 min) and rutin (Rt = 22.9 min) were confirmed in the optimal and cold treated amaranth extracts (Fig. 4.6) by way of comparing their retention times with those of the standard compounds. A small peak of quercetin (Rt = 34.08 min) was present only in the cold treated sample (Fig. 4.6c).The presence of rutin is in agreement with other studies on amaranth flavonoids (Kalinova & Dadakova, 2009; Kraujalis *et al*., 2013).

Various phenolic compounds and flavonoids were reported previously in amaranth seeds and vegetables through HPLC chromatography. For instance, the sprouts of amaranth contained rutin as the main constituent and gallic, p-coumaric and syringic acids as other important constituents (Paśko *et al*., 2008). Kraujalis *et al*. (2013) identified in leaf and flower extracts of *A. hybridus*, rutin, nicotiflorin, isoquercitrin, 4-hydroxybenzoic and p-coumaric acids. It was also shown that the content of polyphenols in different amaranth seed varieties were influenced by many factors, such as genotype, climatic and environmental conditions, Exp sites and seasons

(Steffensen *et al*., 2011). The heat treated sample contains much less compounds and none could be identified by comparing to the known standards used (Fig. 4.6). On the other hand, a large number of recorded peaks on the chromatograms indicate that the extracts are complex mixtures of compounds; however, identification could not be done successfully. Purification of compounds and analysis by NMR and other spectra methods would be necessary for positive identification of minor amaranth constituents.

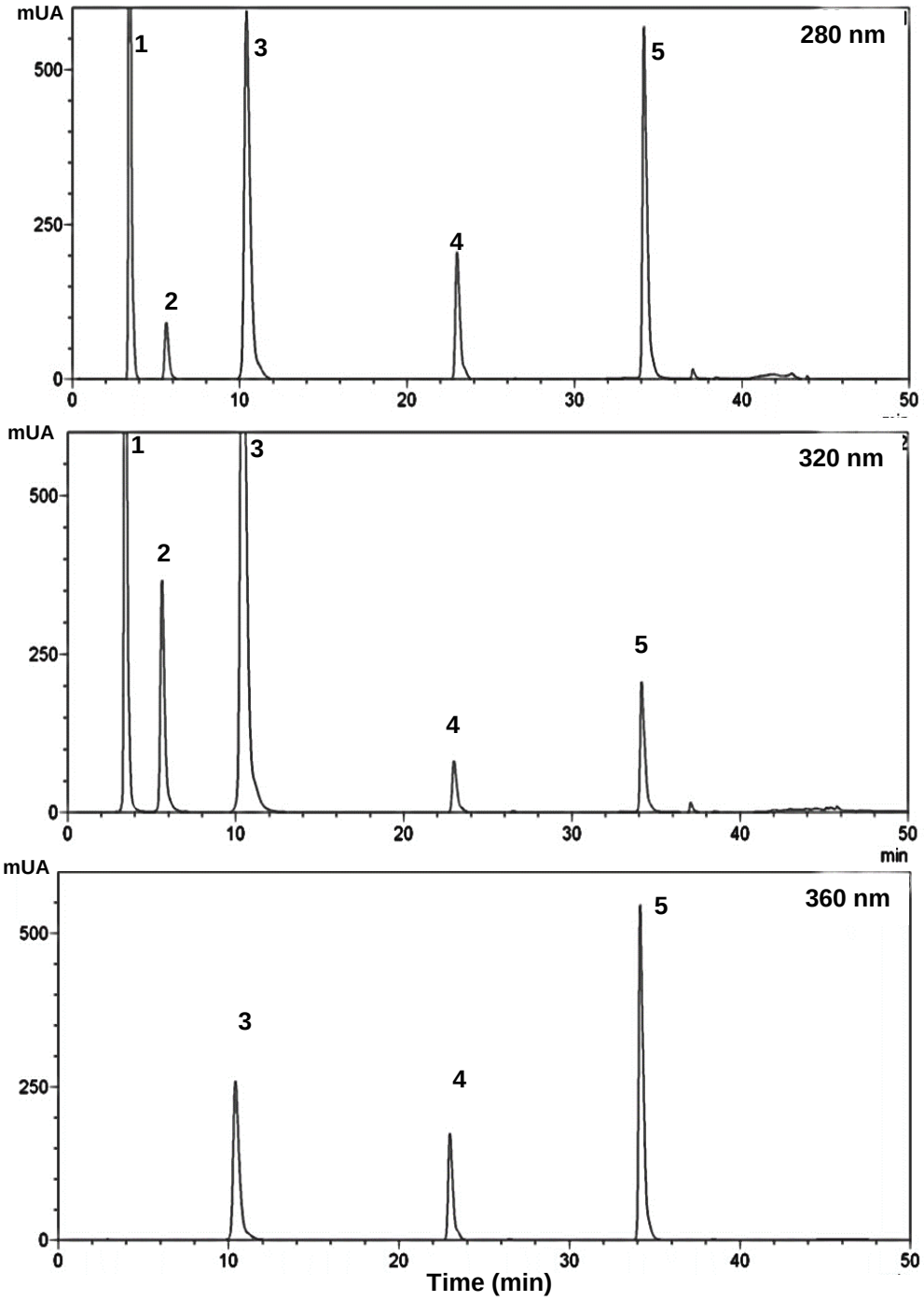


Figure 4.4: HPLC-PDA chromatograms of phenolic standard mixtures detected at 280, 320 and 360 nm. Standards with retentions times (Rt) in minutes: 1 = Gallic acid, Rt 3.56; 2 = Cathechin. Rt 5.99; 3 = Caffeic acid, Rt 10.69; 4 = Rutin, Rt 22.9 and 5 = Quercitin, Rt 34.08.

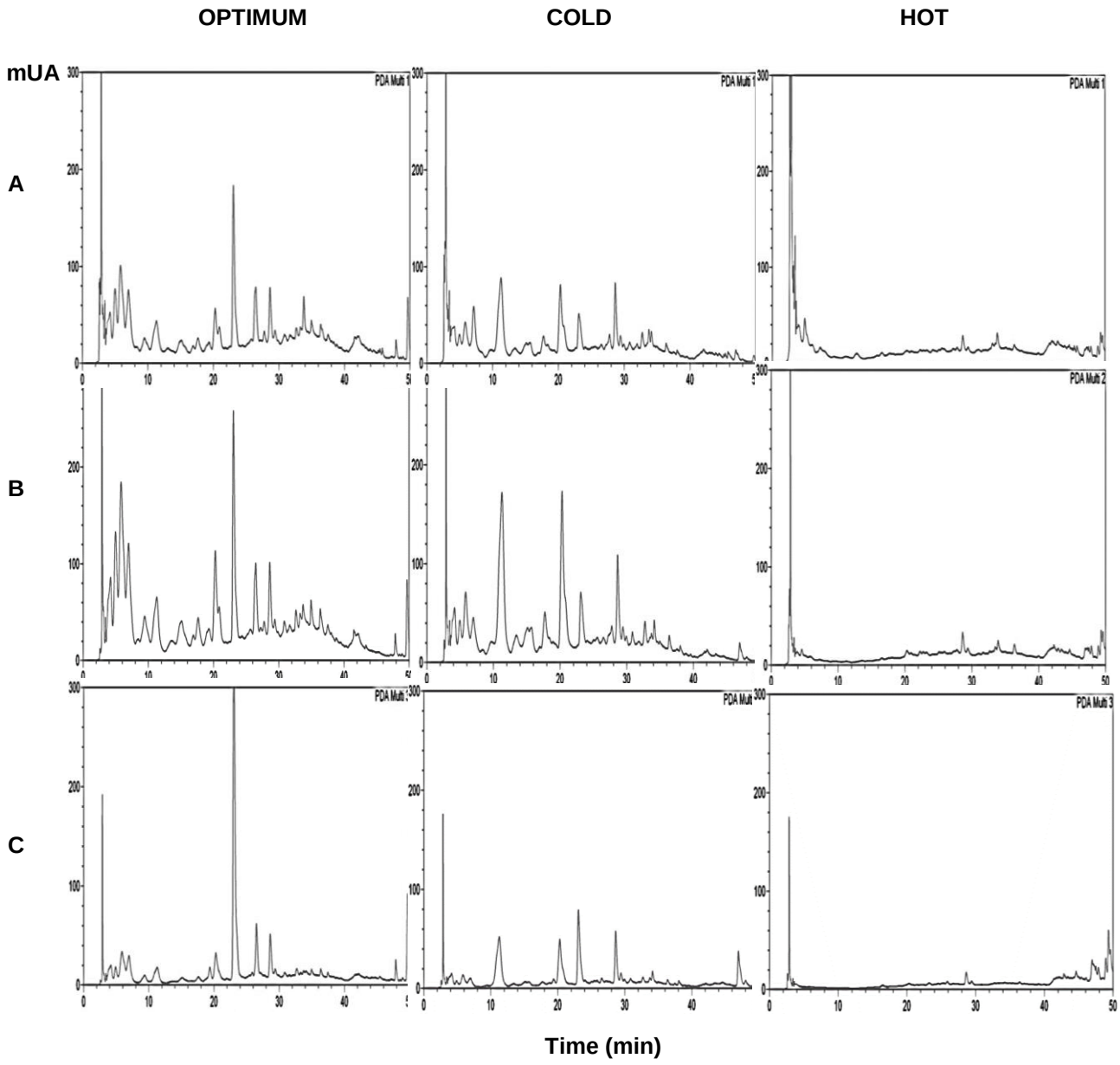


Figure 4.5: Comparison of HPLC-PDA chromatograms of optimal, cold and hot treated *A. cruentus* methanol-water (polar) leaf extracts at 280 nm (A); 320 nm (B); 360 nm (C).

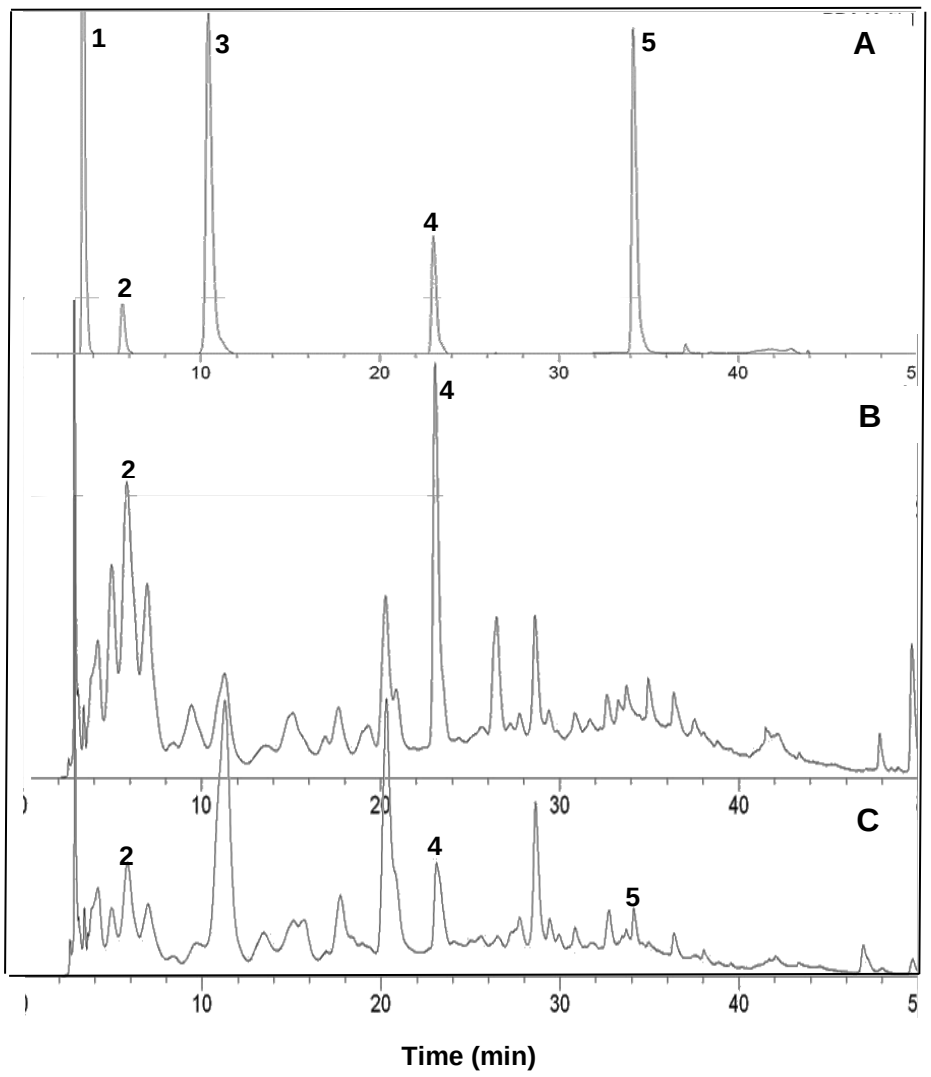


Figure 4.6: HPLC chromatograms of (**A**) phenolic standards, (**B**) optimal and (**C**) cold treated *A. cruentus* methanol-water (polar) leaf extracts.
1 = Gallic acid; **2** = Catechin; **3** = Caffeic acid; **4** = Rutin; **5** = Quercitin.

4.4.3 Gas chromatography and mass spectrometry analysis

Compounds in the non-polar, DCM leaf extracts of the different heat treated plants were separated and identified through gas chromatography and mass spectrometry (GC-MS). With the specific method used, major compounds eluted only after 60 min and made up a total composition of 75.94% (10 compounds), 90.44% (8 compounds) and 91.89% (9 compounds), of the optimal, cold and heat treated samples respectively (Tables 4.7-4.9). From the chromatograms in figure 4.7 the influence of temperature on the expressed compounds in the different extracts are clearly visible. One major compound, comprising 38.59% and eluted at 93.529 min exclusively in the optimum temperature extract, was identified as phosphine imide, P, P, P-triphenyl (Fig 4.8). Zheng *et al.* (2011) identified phosphine imide, P, P, P-triphenyl as one of the major compounds of the seeds of *Dalbergia odorifera*. They previously reported on the heartwood of *D. odorifera*, traditionally used as a kind of Chinese medicine to treat ischemic and cardiovascular diseases.

Neophytadiene and hexadecanoic acid were the only compounds present in all three extracts, although the concentrations of these compounds varied substantially between the treatments (Tables 4.7-4.9). The highest concentration of neophytadiene (27.53%) was found in the cold treated sample, while hexadecanoic acid (13.52%) was maximum in the heat treatment extract. Neophytadiene is a fatty acid-related compound (James *et al.*, 2000) and was identified as a strong antibacterial and anti-inflammatory constituent from the green alga *Dunaliella salina* and *Bursera simaruba* (L.), a plant used in traditional medicine (Carretero *et al*., 2008; Mendiola *et al*., 2008).

Konovalova *et al*. (2013), also reported on the antibacterial properties of neophytadiene, as well as antipyretic, analgesic, anti-inflammatory, antimicrobial and antioxidant activities (Palic *et al*., 2002). Sivakumar & Dhivya (2015) stated that hexadecanoic acid is a fatty acid ester which has antioxidant properties. Hexadecanoic acid is the most common saturated fatty acid found in animals, plants and microorganisms (Gunstone *et al*., 2007). Structural and kinetics studies indicated that it is an inhibitor of phospholipase A, thus, an anti-inflammatory compound (Zhao *et al*., 2005; Aparna *et al*., 2012). Further studies by Aparna *et al*.

(2012) validate the use of medicated oils rich in n-hexadecanoic acid for the treatment of rheumatic symptoms in the traditional medical system.

Five similar compounds were detected in the cold and heat treated plant extracts, however the most abundant compounds were neophytadiene (27.53%) and 9,12,15-octadecatrienoic acid (24.41%) in the cold treatment while the heat treatment extract was rich in dichloroacetic acid, tridec-2-ynyl ester (29.68%; Table 4.4 & 4.5). Alpha-linolenic acid (9,12,15-Octadecatrienoic acid) an n−3 fatty acid, is a member of the group of essential fatty acids (EFAs) found in many seed oils (Seed Oil Fatty Acids – SOFA Database Retrieval). Ingestion of α-linolenic acid has been suggested to have a positive impact on cardiovascular disease (Rodriguez-Leyva *et al*. 2010) and has been reported for anti-bacterial and anti-inflammatory properties (McGaw *et al*., 2009; Yamada, 2009). No information on the bioactivity of dichloroacetic acid, tridec-2-ynyl ester could be found in literature.

Squalene, trans-phytol and the phytosterol, stigmasta-7,22-dien-3-ol were present in both cold and heat treated samples. Squalene was found in high concentrations in oil fractions of *A. caudatus and A. cruentus* (Gamel *et al*., 2007). Squalene is a natural lipid found in the terpenoid family. Squalene acts as a protective agent and has been shown to decrease the side-effects of chemotherapy (Reddy & Couvreur, 2009). This compound enhances the immune response to various antigens and can at a later stage become part of a vaccine (Reddy & Couvreur, 2009). Squalene can reduce cholesterol and triglyceride levels in animals and in humans it might be useful as an addition to certain cholesterol lowering drugs (Kelly, 1999). Studies done on animals show the possibility of anti-cancer properties but no data are available for humans (Kelly, 1999).

Shah (2005) reported on the presence of stigmasta-7,22-dien-3-ol (α-spinasterol) in *A. spinosus.* Alpha-spinasterol is a phytosterol which is found in many plant sources including spinach (Hart & Heyl, 1932). Phytosterols have been shown to reduce the serum total cholesterol and low density lipoprotein (LDL) cholesterol levels in both animal and human studies (Ling & Jones, 1995). In animal studies this compound showed anti-tumor property. Other studies have shown that phytosterols can inhibit the development of colon cancer (Ling & Jones, 1995). Phytosterols are under

preliminary research for their potential to inhibit lung, stomach, ovarian and breast cancers (Woyengo *et al*., 2009).

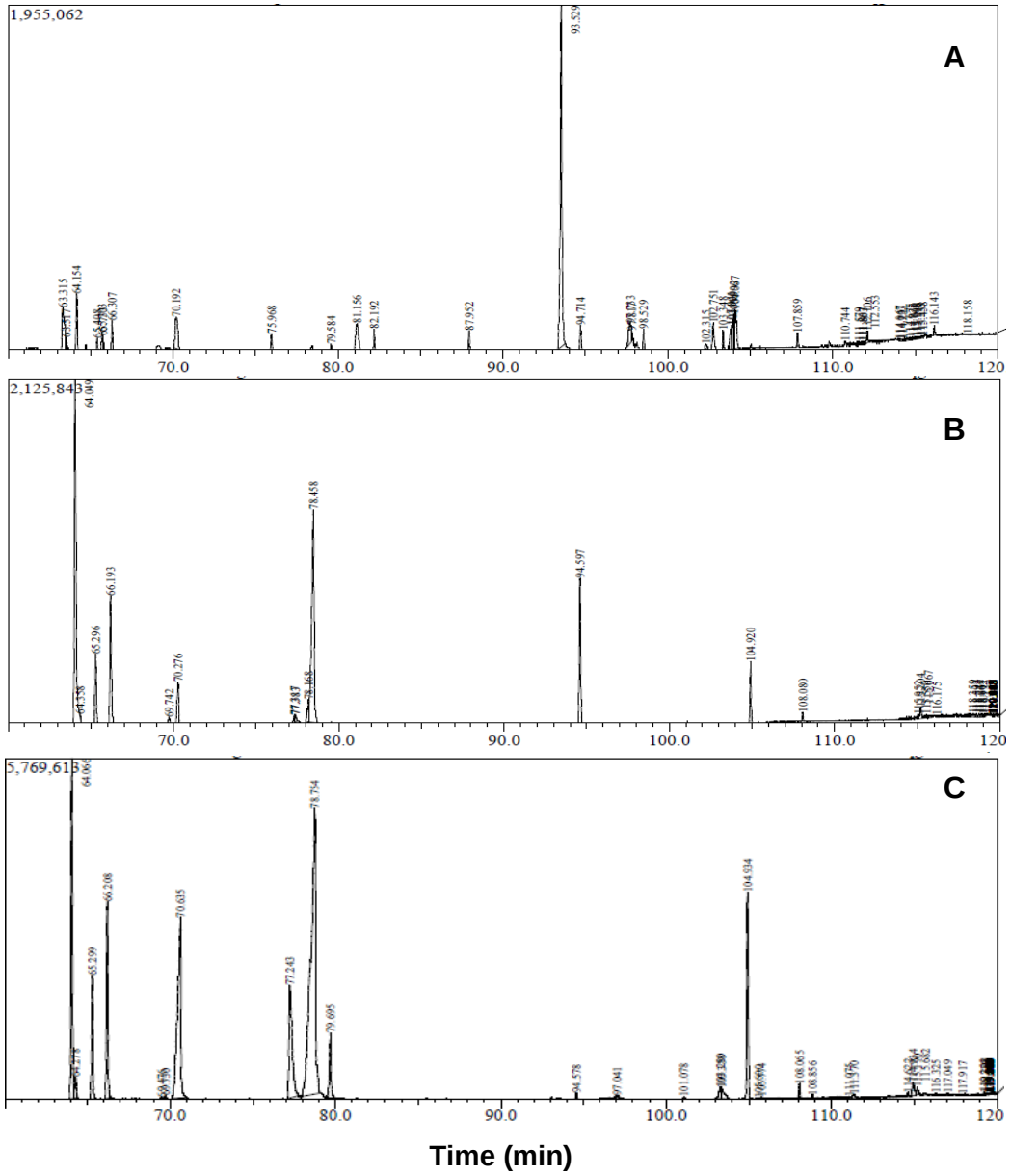


Figure 4.7: GC chromatograms of the DCM extracts of the different temperature treated *A. cruentus* leaf extracts. **A** = Optimal; **B** = Cold; **C** = Hot.

Table 4.7: GC-MS results of compounds present in optimal temperature treated DCM leaf extract of *A. cruentus*.

Retention time (min)	Area %	Compound name*
63.315	6.61	16-Heptadecenal
64.054	4.44	Neophytadiene
70.621	5.42	Hexadecanoic acid (Palmitic acid)
81.156	4.85	Tetrahydrofurano[6a,7a-b]-5-oxa-8-thiaphenanthrene
93.529	38.59	Phosphine imide, P,P,P-triphenyl
97.733	3.48	Diallyldivinylsilane
102.751	2.69	bis-Naphthylfuran
103.841	2.47	Methyl ester of decyclotrenudine
103.992	2.38	(-)-18-Noramborx
104.067	5.01	Benzyl methyl ether
Total	**75.94**	

Area (%) of compound = height of peak x width of peak at ½height x Total area^{-1}

* Identification by MS Library: NIST 05. LIB

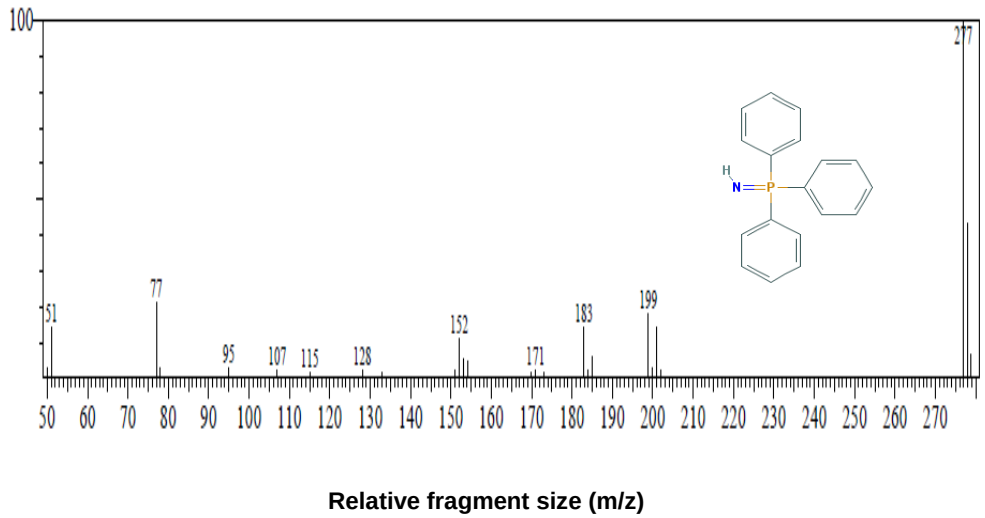


Figure 4.8: MS spectrum and structure of phosphine imide, P,P,P-triphenyl. Spike numbers on the mass spectrum refer to the m/z values of the fragments.

Table 4.8: GC-MS results of compounds present in cold temperature treated DCM leaf extract of *A. cruentus.*

Retention time (min)	Area %	Compound name*
64.049	27.35	Neophytadiene
65.296	4.63	3,7,11,15-Tetramethyl-2-hexadecen-1-ol
66.193	8.94	trans-Phytol
70.676	2.93	Hexadecanoic acid (Palmitic acid)
78.458	24.41	9,12,15-Octadecatrienoic acid (α-Linolenic acid)
94.597	9.87	Di-(2-ethylhexyl)phthalate
104.920	3.32	Squalene
121.002	8.99	Stigmasta-7,22-dien-3-ol (α-Spinasterol)
Total	**90.44**	

Area (%) of compound = height of peak x width of peak at ½height x Total area^{-1}

* Identification by Library: NIST 05. LIB

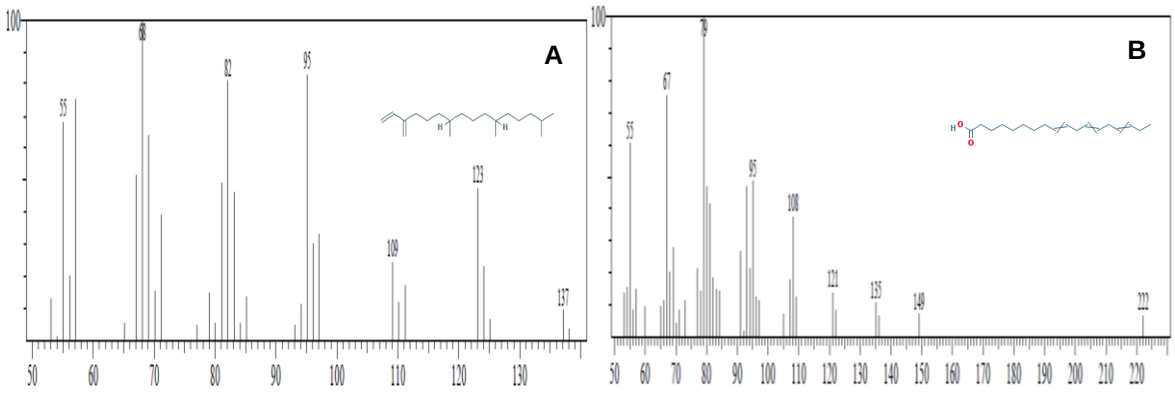


Figure 4.9: MS spectrums and structures of neophytadiene (A) and α-linolenic acid (B). Spike numbers on the mass spectrum refer to the m/z values of the fragments.

Table 4.9: GC-MS results of compounds present in hot temperature treated DCM leaf extract of *A. cruentus*.

Retention time (min)	Area %	Compound name*
64.066	10.26	Neophytadiene
65.299	3.72	3,7,11,15-Tetramethyl-2-hexadecen-1-ol
66.208	6.01	trans-Phytol
70.635	13.52	Hexadecanoic acid (Palmitic acid)
77.243	7.82	2-Hexadecen-1-ol, 3,7,11,15-tetramethyl
78.754	29.68	Dichloroacetic acid, tridec-2-ynyl ester
79.695	2.30	Octadecanoic acid (Stearic acid)
104.934	6.22	Squalene
120.975	12.36	Stigmasta-7,22-dien-3-ol (α-Spinasterol)
Total	**91.89**	

Area (%) of compound = height of peak x width of peak at ½height ≤x Total area^{-1}

* Identification by Library: NIST 05. LIB

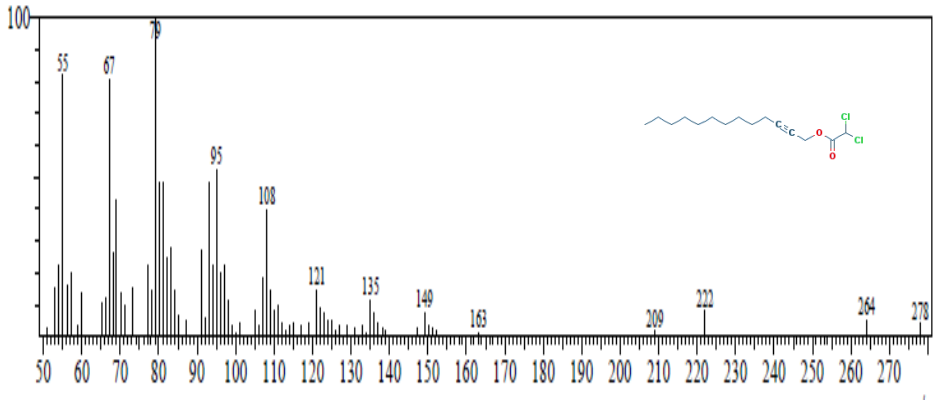


Figure 4.10: MS spectrum and structure of dichloroacetic acid, tridec-2-ynyl ester. Spike numbers on the mass spectrum refer to the m/z values of the fragments.

The variety of compounds identified in the methanol-water and DCM extracts of *A. cruentus* leaves under different temperature regimes confirmed the medicinal value of amaranth, however no information could be found on the influence of these compounds on other plants. Due to the different biological activities of these compounds it might be possible that these extracts contain biologically active allelochemical compounds. Further studies are needed to determine the allelopatic activities of these extracts.

4.5 Allelopathy

4.5.1 Phytotoxicity of plant litter

Plant litter refers to the ground leaf material of *A. cruentus* plants exposed to different temperature treatments.

4.5.1.1 Germination

Crop farmers require a good germination percentage and seedling establishment as this will determine the ultimate yield of the crops. One of the main influences that allelopathic plants have on succeeding plants is to inhibit germination of the new plant's seeds. The plant litter accumulation may cause indirect chemical effects mediated by the release of allelochemicals into the environment after its decomposition (Bonanomi *et al*., 2006). When grown in the same soil different plants will react differently when exposed to allelopathic plant litter (Qasem, 2010).

Analysis from our study indicated that germination percentage was significantly affected by growth temperatures (T) of the amaranth ($P<0.0001$), litter concentration (C) ($P<0.0001$), vegetable type (V) ($P<0.0001$), the T×V interaction (P-0.0041) and V×C interaction ($P<0.0001$). The T×V×C (P-0.0540) interaction showed no statistical differences.

As can be seen from Fig. 4.11 there were highly significant ($P<0.01$) differences in the reaction of the various vegetables to the litter of amaranth plants grown under stress or optimal temperature (temperature stress control) conditions.

Vegetables that were exposed to plant litter of heat treated amaranth plants showed the most significant germination inhibition (Fig. 4.11). Pepper was the most sensitive

when exposed to litter of amaranth grown under high temperature conditions, with 60% germination inhibition, followed by cucumber at 50%. Lettuce germination was the least affected with an inhibition of only 22%, while the germination of tomato seeds was reduced by 40%. Interestingly, the germination percentage of lettuce seed was more negatively affected by amaranth litter from plants cultivated at optimal temperatures than by litter from plants grown at both cold and hot temperatures. It appeared as though cucumber, pepper and tomato seeds were generally more sensitive to amaranth litter than lettuce.

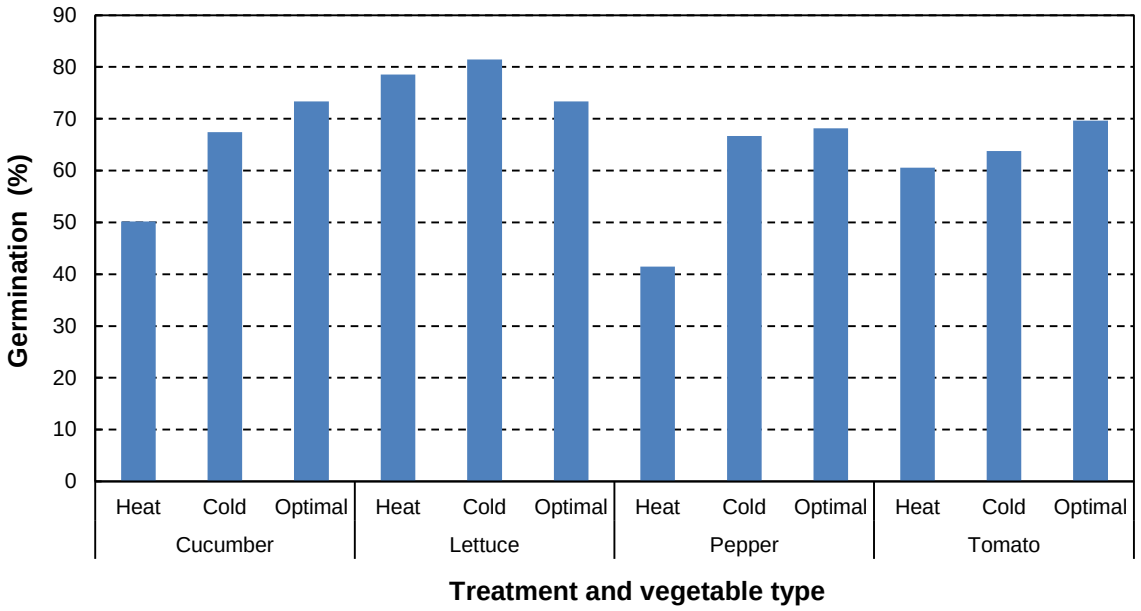


Figure 4.11: Effect of amaranth litter from plants grown at different temperatures on the germination percentage of various vegetables. $P<0.01$ ($LSD_{(T\leq0.05)}$ = 2.71). n = 96.

The significant interaction between amaranth litter concentration and the four vegetable species is illustrated in Fig. 4.12. Here it can be seen that the germination percentage of all four vegetables decreased significantly with increased concentration of amaranth litter that was added to the growth medium. However the degree of inhibition differed between the four vegetables used.

Peppers were the most sensitive to amaranth litter with the germination percentage dropping from 100% through 54% to 22% at the 0, 1 and 5 mg mL^{-1} litter concentrations respectively. Tomatoes were the least affected, with germination

inhibited by only 17% and 43%, followed by 19% and 33% for lettuce and 36 and 66% for cucumber at 1 and 5 mg mL^{-1}.

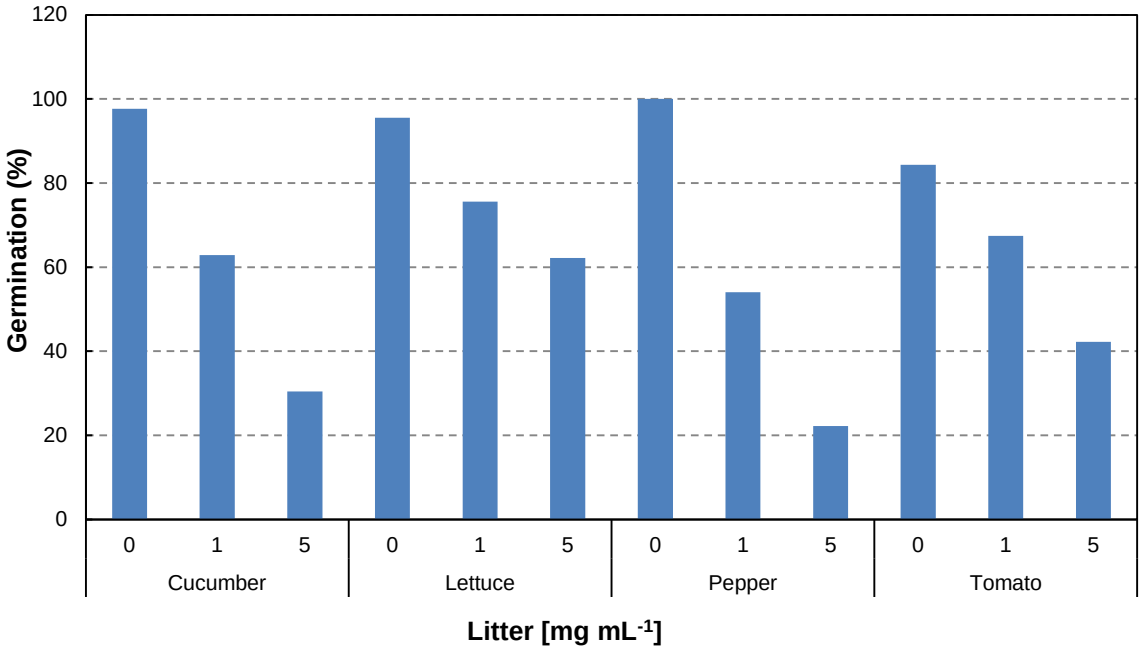


Figure 4.12: Effect of amaranth litter concentration on the germination percentage of four vegetables ($LSD_{(T\leq0.05)}$ = 2.71). n = 96.

Vegetable susceptibility varies from peppers which are highly affected followed by cucumber, tomato and lettuce being the least susceptible to the plant litter. When vegetables are used in a rotation system it is not advisable to plant peppers after a crop of *A. cruentus* plants.

Germination of all vegetables was adversely affected by both concentrations of amaranth litter, irrespective of the temperature treatment to which the source plants were exposed to (Fig. 4.13). Manikandan & Prabhakaran (2014) also found adverse effects on seed germination of Green Gram mung beans when exposed to allelopathic weed litter. From Fig 4.13 it can be seen that the litter from heat treated amaranth plants caused the greatest decline in germination percentage of lettuce, 43% and 74% at the 1 and 5 mg concentrations respectively. At the 1 mg mL^{-1} a reduction of 24% was caused by the optimal growth temperature litter, though the

reduction of 23% caused by cold produced litter was only just significantly less than that caused by the optimal growth temperature litter.

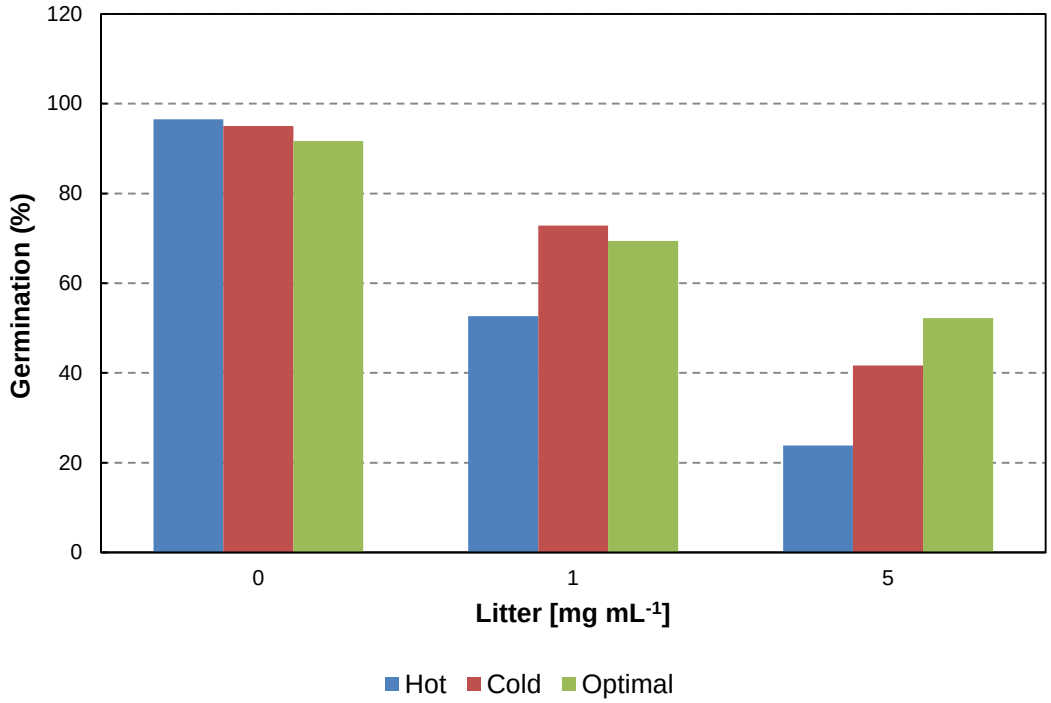


Figure 4.13: Effect of litter concentration from amaranth plants grown at different temperatures on germination of vegetables. $P<0.01$ ($LSD_{(T\leq0.05)}$ = 2.73). n = 96.

At 5 mg mL^{-1} significant differences in germination percentage between the three temperatures treatments was found, with highest inhibition caused by the stressed samples.

These results indicate that the allelopathic compounds affecting germination are produced by amaranth irrespective of growth temperature although the litter of plants grown under stressful conditions (hot or cold) have a greater allelopathic effect than those at optimal conditions.

Allelopathy plays a significant role in seed dormancy, seed germination and seedling emergence (Qasem, 2010). The influence of allelochemicals on the balance of plant populations and species stability, microorganisms, natural enemies and insect populations, and the spread of pathogens is another important role of allelopathy (Qasem, 2010).

Results show that it is vital to understand under which conditions amaranth was cultivated. Understanding this is important in order to determine which vegetable is best suited to be grown in that soil as a succeeding crop in a rotation system.

4.5.1.2 Hypocotyl

A difference in interaction of the vegetables towards concentration of plant litter and temperature treatments was noticed. Incorporation of 1 mg mL^{-1} amaranth litter with the growth medium resulted in significant decreases in the hypocotyl lengths of cucumber, pepper and tomato (Fig. 4.14). In cucumber (Fig. 4.14 A), pepper (Fig. 4.14 B) and tomato (Fig. 4.14 C) a reduction of 37%, 76% and 78% respectively in hypocotyl length was found. Increasing the litter concentration to 5 mg mL^{-1} did not enhance the reduction in hypocotyl length for either cucumber or pepper. However a 78% greater reduction was found in tomato, indicating that tomatoes were more sensitive to increasing concentrations than the other two species.

ANOVA of growth data from cucumbers showed that the hypocotyl length was significantly affected by exposure to litter of amaranth plants grown at different temperature regimes (Fig 4.15). These differences were, however, found not to be significant when compared by means of Tukey's test at the 5% level of significance. This is probably due to Tukey's test being far stricter than Fisher's test. The other vegetables did not show significant differences in hypocotyl length at the various temperatures.

It does, however, show that there was a significant trend, with increasing levels of allelopathy being found as the temperature under which the amaranth plants was grown increased. This trend can be seen to accelerate as the temperature increases beyond the optimal temperatures for amaranth growth.

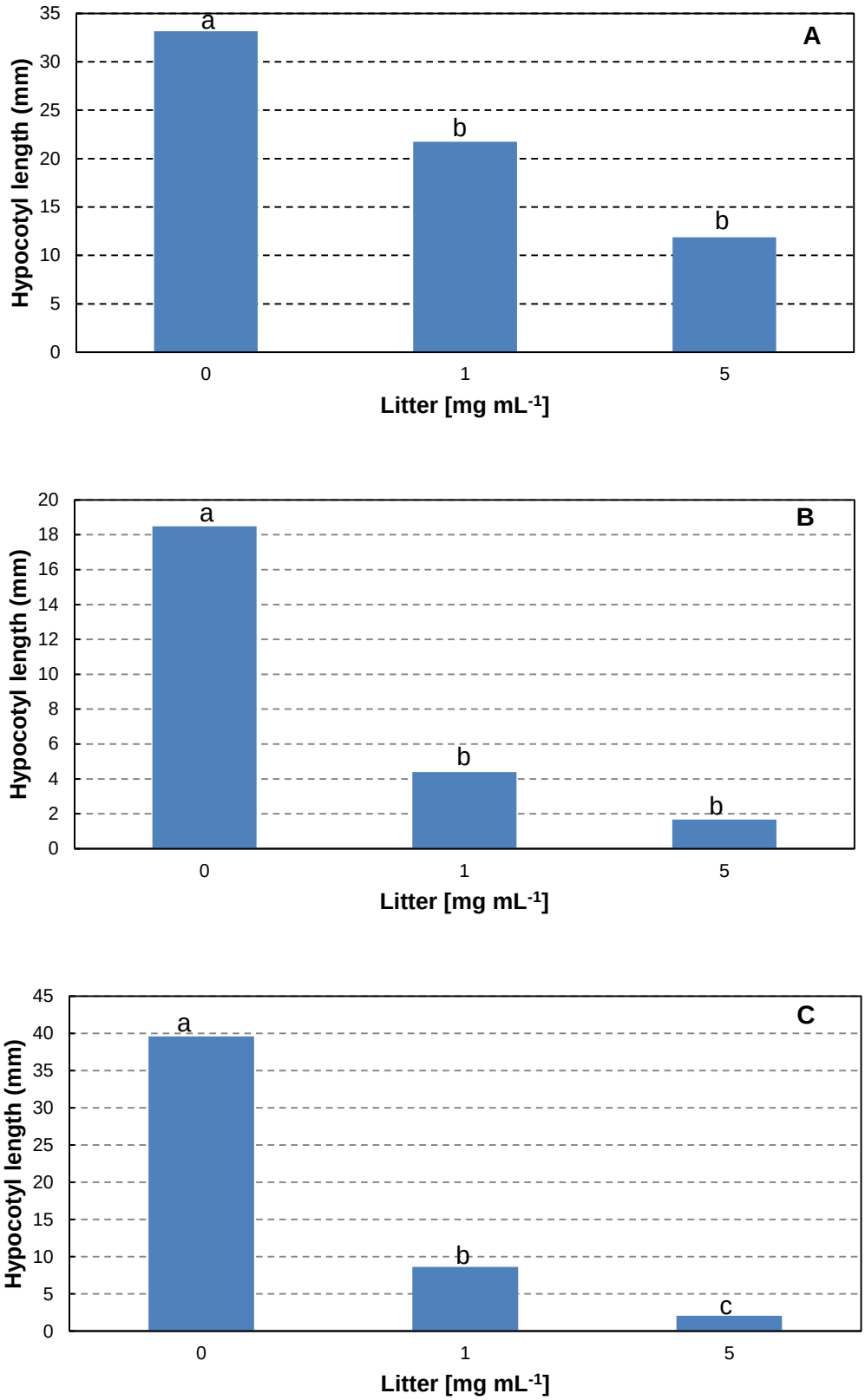


Figure 4.14: Effect of amaranth litter at different concentrations on the hypocotyl length (mm) of various vegetables. Cucumber (A): $LSD_{(T\leq0.05)}$ = 11.06; Pepper (B): $LSD_{(T\leq0.05)}$ = 4.44; Tomato (C): $LSD_{(T\leq0.05)}$ = 4.67. n = 13.

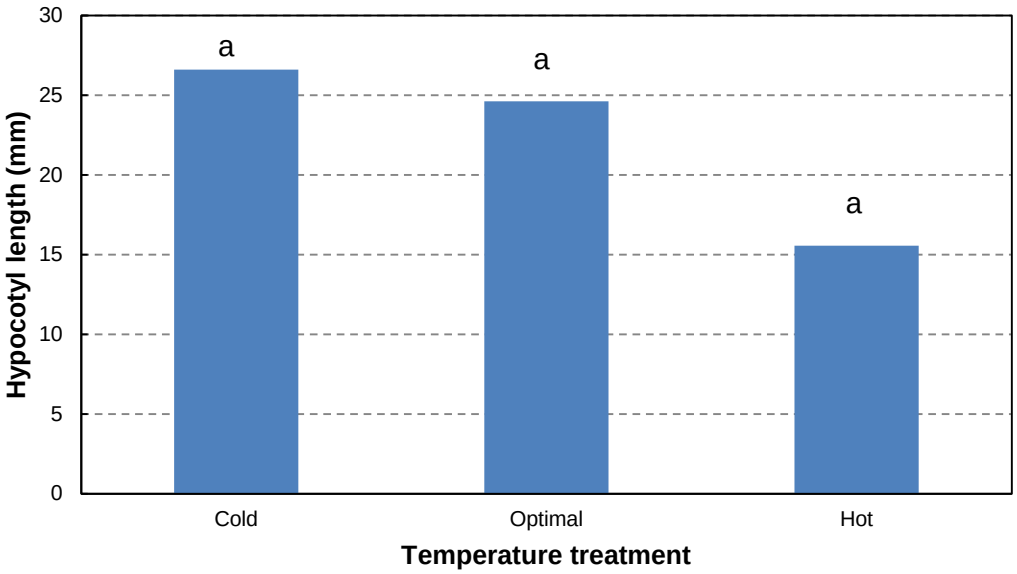


Figure 4.15: Average hypocotyl length of cucumber when exposed to the different temperature treatments. $LSD_{(T\leq0.05)}$ = 11.06. n = 13

In lettuce there was a two way interaction between concentration of plant litter and the temperature treatment of the plants (Fig 4.16). Concentration and temperature treatments were highly significant ($LSD_{(T\leq0.05)}$ = 3.43) in reducing hypocotyl length. At 5 mg mL^{-1} the heat treatment displayed a reduction of 17.38 mm to 1.33 mm (92%), cold treatment 13.14 mm to 6.69 mm (49%) and the optimal treatment the length was reduced from 8.05 mm to 2.23 mm (72%) (Fig. 4.16). It is clear that lettuce seedlings were mostly affected by the heat treated plant litter at the highest concentration with the overall reduction in hypocotyl length of 92%.

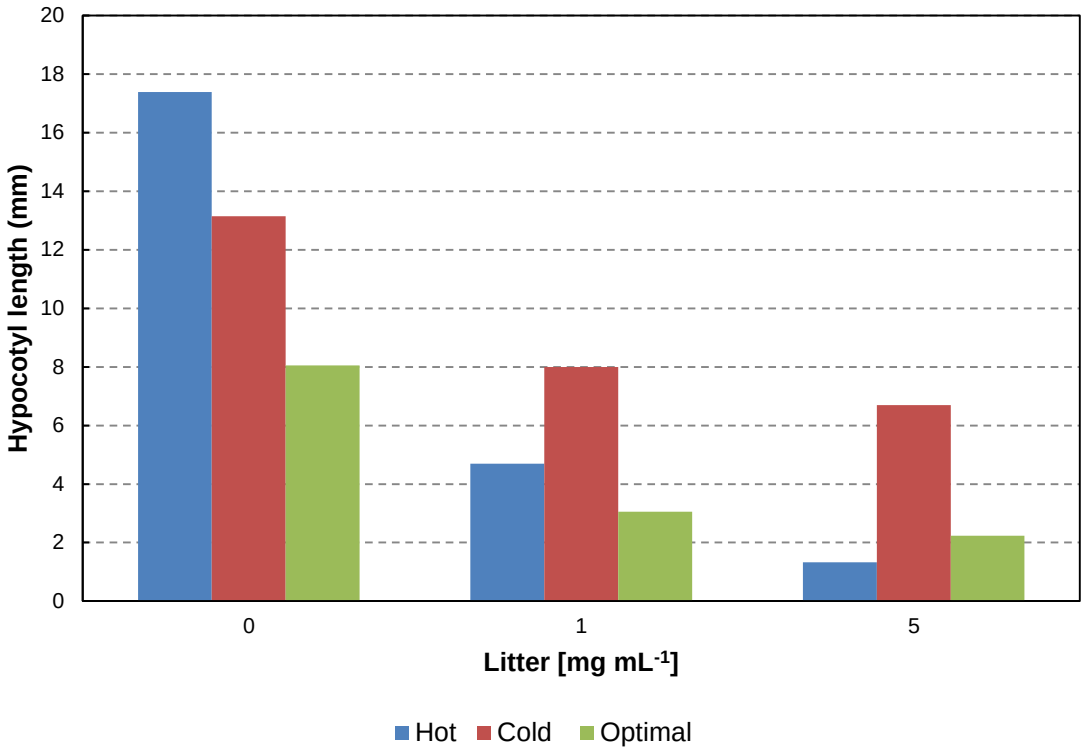


Figure 4.16: Two way interaction of hypocotyl length of lettuce seedlings. $LSD_{(T \leq 0.05)}$ = 3.43. n = 13.

4.5.1.3 Radicle

Radicle (root) growth of cucumber, pepper and tomato were significantly influenced by the concentration of the treated plant litter (Fig. 4.17). At 5 mg mL^{-1} the reduction in radicle growth was 63%, 91% and 91% for cucumber (Fig. 4.17 A), pepper (Fig. 4.17 B) and tomato (Fig. 4.17 C) respectively. All three concentrations affected radicle growth significantly in cucumber and pepper, while no significant difference between 1 and 5 mg mL^{-1} was noted for tomato and the radicle was already severely inhibited by 85% at 1 mg mL^{-1}.

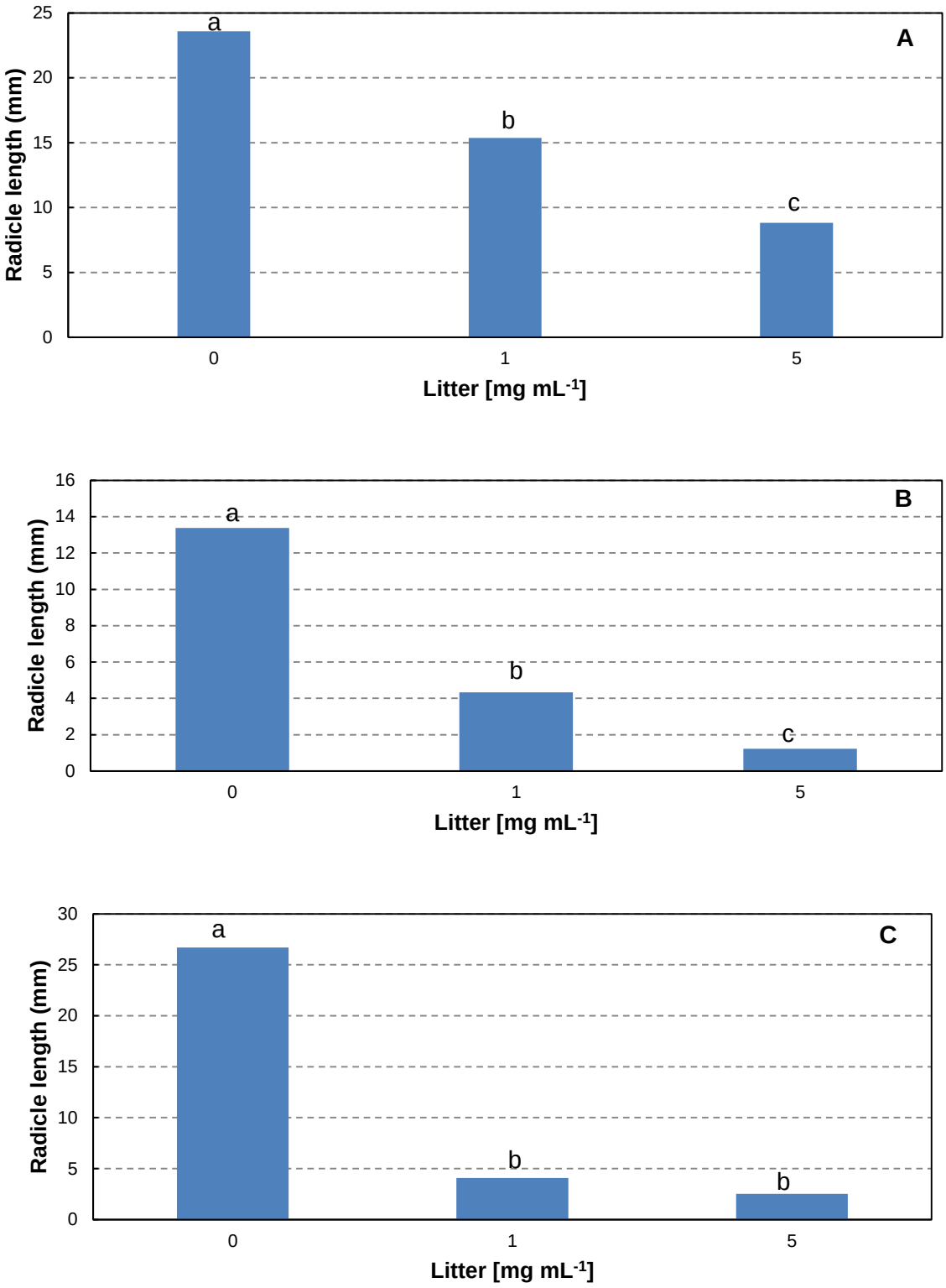


Figure 4.17: Effect of amaranth litter at different concentrations on the average radicle length of various vegetables. Cucumber (A): $LSD_{(T\leq0.05)}$ = 6.45; Pepper (B): $LSD_{(T\leq0.05)}$ = 2.33; Tomato (C): $LSD_{(T\leq0.05)}$ = 2.28. n = 13.

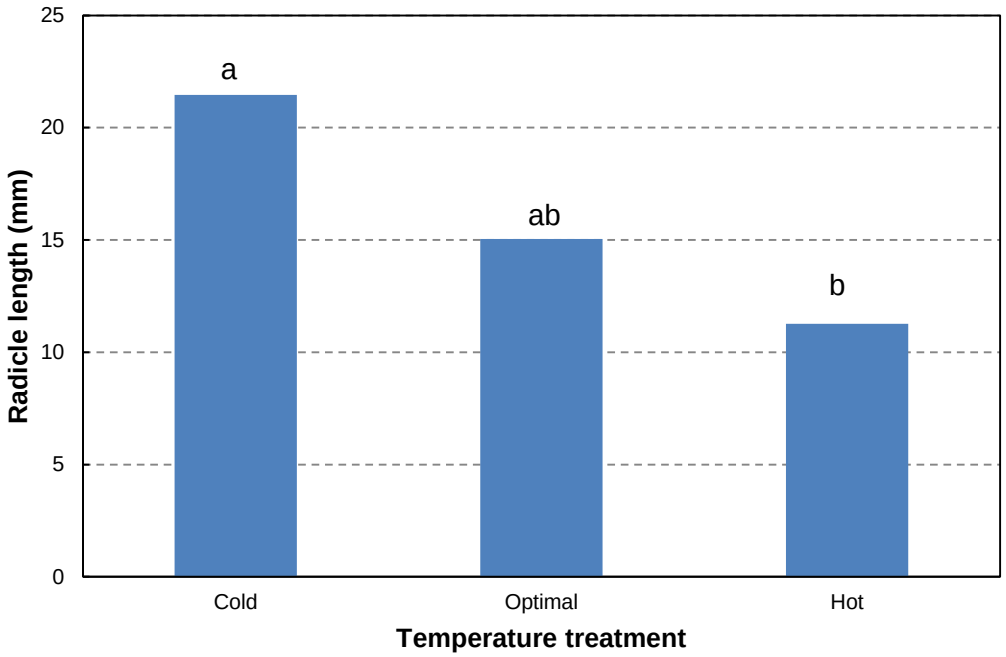


Figure 4.18: Average radicle length of cucumber when exposed to the different temperature treatments. $LSD_{(T \leq 0.05)}$ = 6.45. n = 13.

Comparable to the results found with the hypocotyl, the cucumber radicles had a significant interaction with the different temperature treatments (Fig. 4.18), and lettuce showed a two way interaction between concentration of plant litter and the temperature treatment (Fig. 4.19). Concentration and temperature treatments were highly significant in reducing radicle length ($LSD_{(T \leq 0.05)}$ = 3.41). At 1 mg mL^{-1} there was a reduction in the radicle length of lettuce from 26.69 mm to 8.31 mm (69%), 36.77 mm to 15.46 mm (58%) and 23.69 mm to 4.85 mm (79%) for heat, cold and optimal temperature treatments respectively. The 5 mg mL^{-1} concentrations of all the treatments inhibited radicle development almost completely.

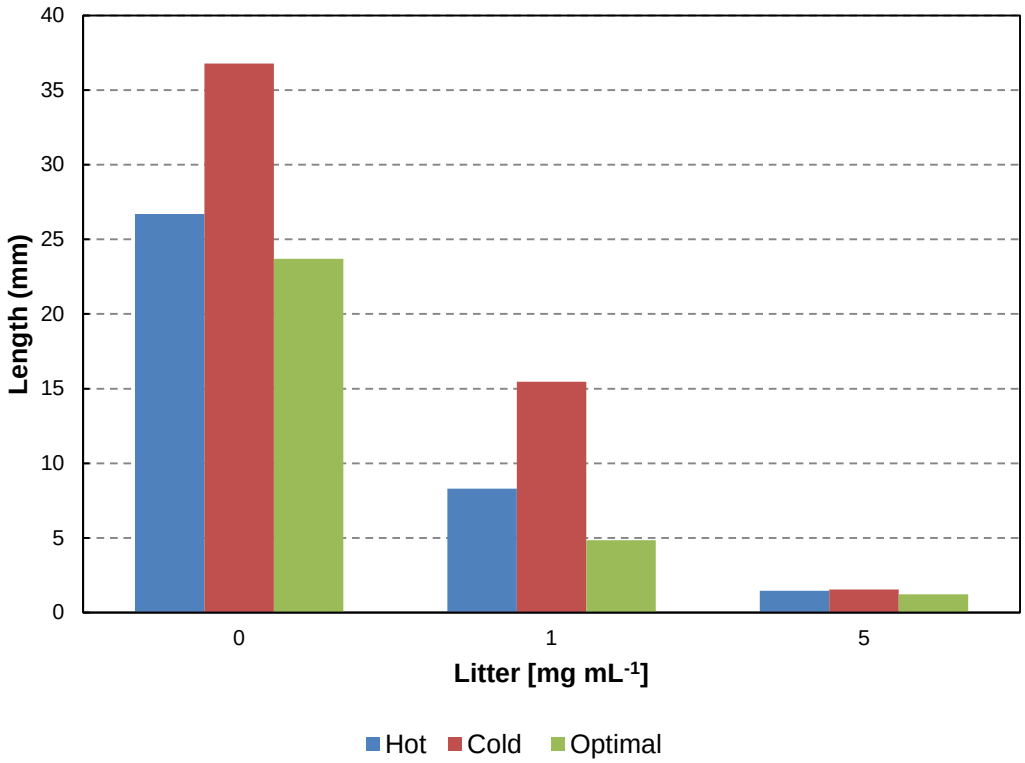


Figure 4.19: Two way interaction of radicle length of lettuce seedlings. $LSD_{(T\leq0.05)}$ = 3.41.

From the *in vitro* bioassay a connotation between the inhibition of the radicle and hypocotyl was noted. Once the radicle (root) has been damaged the uptake of nutrients becomes harder and because of this the growth of the hypocotyl (shoot) will be adversely effected. Manikandan & Prabhakaran (2014) specified that plants with poorly developed root systems struggle to absorb nutrients as well as anchoring the plant in the soil, which have an effect on growth and development. Menges (1988) found similar results where dried stem tissues of Palmer amaranth severely inhibited the growth of grain sorghum organ length. Grand Slam cultivar cabbage had root growth inhibition when exposed to Palmer amaranth (Menges, 1988). It is clear that allelopathy affects the seeds at an early stage. Tabriza & Yarnia, (2011) stated that allelopathic compounds disrupt the mechanism of DNA replication, photosynthetic and mitochondrial function which in turn affects the absorption of ions and water. They found that maize was adversely affected when exposed to pigweed extracts.

Results by Quayyam *et al*. (2000) on extracts from *Cyperus rotundus,* as well as the leachate of the leaves and tubers, showed significant reduction in the germination

and growth of rice. This plant is a well-known weed and just like Amaranth it causes a lot of problems in agriculture. Sadaqa *et al*. (2010), found that *A*. *graecizans* inhibited the germination and growth of onions in pot trials. Inhibition of onion height and root length was caused by *A. graecizans* foliage while root exudates were most inhibitory to seed germination.

It is known that some phenolic compounds and flavonoids have allelopathic properties (Inderjit *et al*., 1995; Bais *et al*., 2003; Khan *et al*., 2009), however our results indicate that the heat treatment which contained the least TPC and flavonoids (Table 4.3), was the most detrimental in germination, hypocotyl and radicle development, indicating the possible expression of other phytotoxic compounds during heat stress.

4.5.2 Phytotoxicity of extracts

The solvents, DCM and methanol-water (70:30), used for extraction had varying polarities with different chemical properties, therefore provide a broad spectrum of phytochemicals that could be extracted from the different treatments of *A. cruentus* leaves. There are many classes of allelopathic compounds including terpenes and phenols that are naturally produced by many plants (Jefferson & Pennacchio, 2003). Terpenes and phenols are normally produced in the leaves and when the leaves drop to the ground and decay, these allelopathic chemicals are released into the soil (Jefferson & Pennacchio, 2003).

4.5.2.1 Germination

Germination of all the vegetables were significantly inhibited ($LSD_{(T\leq0.05)} = 1.88$) when exposed to different concentrations of extracts of the different temperature treatments (Table 4.10). Peppers were the most sensitive when exposed to the methanol-water extract of the optimal temperature treatment with an inhibition of 91% at 0.5 mg mL^{-1}, while the DCM extract of the cold temperature treatment resulted in the main germination inhibition of peppers. Germination of tomato seeds were significantly inhibited at all concentrations and treatments, with no significant differences between the polar and non-polar extracts. Lettuce seed germination at 2 mg mL^{-1} showed an inhibition of more than 60% for all the treatments. Cucumber was the least affected by the methanol-water extract compared to the other

vegetables, while the DCM extract of the optimal treatment showed the highest inhibition (80%) at 2 mg mL^{-1}. Germination was progressively inhibited with an increase in extract concentration except for cucumber when exposed to methanol-water extracts from the optimal and heat treatments (Table 4.10).

Table 4.10: Germination percentage of different vegetable seeds exposed to increasing concentrations of MeOH-H_2O and DCM leaf extracts of *A. cruentus* grown at optimal, cold and hot temperatures.

Extract [mg mL^{-1}]	**Germination % of Cucumber**			
	Temperature treatment			
MeOH-H_2O	Optimal	Cold	Hot	Ave
0	100	100	100	100
0.5	61	66	75	67
2	84	45	84	71
Ave	82	70	86	
DCM				
0	100	100	100	100
0.5	44	96	49	63
2	20	42	49	37
Ave	55	79	66	

Extract [mg mL^{-1}]	**Germination % of Lettuce**			
	Temperature treatment			
MeOH-H_2O	Optimal	Cold	Hot	Ave
0	100	100	100	100
0.5	24	29	31	28
2	24	24	36	28
Ave	49	51	56	
DCM				
0	100	100	100	100
0.5	69	67	42	59
2	42	24	36	34
Ave	70	64	59	

Extract [mg mL^{-1}]	**Germination % of Pepper**			
	Temperature treatment			
MeOH-H_2O	Optimal	Cold	Hot	Ave
0	100	100	100	100
0.5	9	24	29	21
2	9	24	33	22
Ave	39	49	54	
DCM				
0	100	100	100	100
0.5	49	29	56	44
2	40	22	47	36
Ave	63	50	68	

Extract [mg mL^{-1}]	**Germination % Tomato**			
	Temperature treatment			
MeOH-H_2O	Optimal	Cold	Hot	Ave
0	100	100	100	100
0.5	78	67	64	70
2	31	36	33	33
Ave	70	68	66	
DCM				
0	100	100	100	100
0.5	78	64	69	70
2	31	36	42	36
Ave	70	67	70	

$LSD_{(T \leq 0.05)} = 1.88$. n = 96.

4.5.2.2 Hypocotyl

Only lettuce was not affected by the methanol-water extract at the lower concentration (0.5 mg mL^{-1}), while both polar and non-polar extracts significantly reduced hypocotyl length of all the vegetables at 2 mg mL^{-1} concentration (Table 4.11). Significant differences between the 0, 0.5 and 2 mg mL^{-1} concentrations occurred only in tomato exposed to the DCM extract. Methanol-water extracts (2 mg mL^{-1}) caused the most prevalent reduction (84%) in hypocotyl lengths of cucumber (31.69 to 5.05 mm) and pepper (20.23 to 3.23 mm), while in the case of 2 mg mL^{-1} DCM extract, the biggest decrease occurred in tomato hypocotyl (38.23 to 5.54 mm = 85%).

No significant differences in hypocotyl length of all the vegetables were detected between different temperature treatments (T = ns) of the polar extracts, however the cold and heat treatment DCM extracts, significantly inhibited hypocotyl length of cucumber (T = 11.15) and lettuce (T = 4.89; Table 4.11). These results indicate that cucumber and lettuce were more sensitive to allelopathic compounds induced in amaranth plants during stress temperatures.

Results by Amini (2009; 2013), proved that root exudates of *A. retroflexus* had inhibitory effects on shoot length of both crop (wheat) and vegetable (common bean) plants. Konstantinović *et al*. (2014), found that aqueous extracts from the leaves, roots and stems of *A. retroflexus* had inhibitory effects on the hypocotyl growth of maize.

Table 4.11: Hypocotyl length of different vegetable seeds exposed to increasing concentrations of MeOH-H_2O and DCM leaf extracts of *A. cruentus* grown at optimal, cold and hot temperatures.

Extract [mg mL^{-1}]	Hypocotyl length of Cucumber (mm)			
MeOH-H_2O	**Temperature Treatment**			
	Optimal	**Cold**	**Hot**	**AVG**
0	31.69±30.77	31.69±30.77	31.69±30.77	**31.69 a**
0.5	7.92±4.09	8.08±3.45	25±12.29	**13.67 b**
2	3.54±3.31	1.85±1.82	9.77±13.18	**5.05 b**
AVG	14.38	13.87	22.15	
$LSD_{(T\leq0.05)}$	**T=ns**	**C=9.41**	**T×C=ns**	
DCM				
0	35.38±25.16	43.85±29.91	35.38±25.16	**38.20 a**
0.5	32.15±20.33	24±19.24	13.46±13.36	**23.20 b**
2	38.23±23.10	13.77±16.05	12±15.79	**21.33 b**
AVG	35.25	27.21	20.28	
$LSD_{(T\leq0.05)}$	**T=11.15**	**C=11.15**	**T×C=ns**	

Extract [mg mL^{-1}]	Hypocotyl length of Lettuce (mm)			
MeOH-H_2O	**Temperature treatment**			
	Optimal	**Cold**	**Hot**	**AVG**
0	28.77±9.07	28.77±9.07	28.77±9.07	**28.77 a**
0.5	26.77±16.50	29.15±17.83	31.69±11.46	**29.03 a**
2	20.62±12.80	6.31±9.46	13.08±7.99	**13.34 b**
AVG	25.39	21.41	24.51	
$LSD_{(T\leq0.05)}$	**T=ns**	**C=6.23**	**T×C=ns**	
DCM				
0	33.77±12.99	37.23±10.48	35.46±11.71	**35.49 a**
0.5	28.85±6.27	17.31±9.29	9.77±4.64	**18.64 b**
2	26.77±7.93	7.08±8.75	25.38±7.79	**19.74 b**
AVG	29.79	20.54	23.54	
$LSD_{(T\leq0.05)}$	**T=4.89**	**C=4.89**	**T×C=4.89**	

Extract [mg mL-1]	Hypocotyl length of Pepper (mm)			
MeOH-H2O	**Temperature Treatment**			
	Optimal	**Cold**	**Hot**	**AVG**
0	20.23±15.34	20.23±15.34	20.23±15.34	**20.23 a**
0.5	2.69±4.55	5.62±3.91	11.31±7.78	**6.54 b**
2	1.08±1.80	3.46±2.26	5.15±1.99	**3.23 b**
AVG	8	9.77	12.23	
$LSD_{(T\leq0.05)}$	**T=ns**	**C=4.35**	**T×C=ns**	
DCM				
0	16.08±6.21	16.08±6.21	16.08±6.21	**16.08 a**
0.5	6.31±2.56	4.31±5.75	2.46±1.61	**4.36 b**
2	3.69±2.66	4.31±5.02	8.77±7.33	**5.59 b**
AVG	8.69	8.23	9.10	
$LSD_{(T\leq0.05)}$	**T=ns**	**C=2.78**	**T×C=2.78**	

Extract [mg mL^{-1}]	Hypocotyl length of Tomato (mm)			
MeOH-H2O	**Temperature Treatment**			
	Optimal	**Cold**	**Hot**	**AVG**
0	38.23±24.53	38.23±24.53	38.23±24.53	**38.23 a**
0.5	13.54±7.18	5.23±3.19	10.46±4.63	**9.74 b**
2	9.69±3.90	17±15.22	11.38±9.27	**12.69 b**
AVG	20.45	20.15	20.02	
$LSD_{(T\leq0.05)}$	**T=ns**	**C=3.83**	**T×C=3.83**	
DCM				
0	38.23±24.53	38.23±24.53	38.23±24.53	**38.23 a**
0.5	12.54±10.04	22.26±16.61	11.77±7.38	**15.64 b**
2	7.46±8.32	4.46±2.50	4.69±2.75	**5.54 c**
AVG	19.41	21.77	18.23	
$LSD_{(T\leq0.05)}$	**T=ns**	**C=8.01**	**T×C=8.01**	

Different letters along the column indicate significant differences at T≤0.05 (Tukey test). Significant differences within each vegetable; N = 13.

4.5.2.3 Radicle

Tomato was the only vegetable not affected by the methanol-water extract at the lower concentration (0.5 mg ml^{-1}), while both polar and non-polar extracts significantly reduced radicle length of all the vegetables at 2 mg mL^{-1} concentration (Table 4.12). Significant differences between 0, 0.5, 2 mg mL^{-1} concentrations occurred in both lettuce and pepper when exposed to methanol-water extract. The higest concentration of the methanol-water extract (2 mg mL^{-1}) caused the most severe reduction (80%, 81% & 88%) in radicle lengths of cucumber (24 mm to 4.82mm), lettuce (26.31 mm to 5 mm) and pepper (13.92 mm to 1.67 mm) respectively. The DCM extract at 2 mg mL^{-1} affected radicle length of peppers most severely (24.38 mm to 5.77 mm = 76%).

No significant differences in radicle length of all the vegetables were detected between the different temperature treatments (T = ns) of the non-polar DCM extracts, however the optimal treatment of the methanol-water extracts, significantly inhibited radicle length of pepper (T = 2.29; Table 4.12). These results indicated that pepper and tomato, both Solanaceae, were the most sensitive to the allelopathic compounds that were produced in amaranth plants during stress treatments. It was observed that in all the affected vegetables the roots had severe damage, turning brown and starting to die off. When the roots are damaged it becomes difficult for the seedling to extract any water or nutrients from the substrate. When a plant is unable to take up water or nutrients it will start to die and this will cause loss in yield. Studies on soil will also help to see if the effect will coincide with the results found in the laboratory.

Many studies have been done on allelopathy of the polar extracts of amaranth, but no information is available on the non-polar compounds. Obaid & Qasem (2005) found that *A. gracilis* had inhibitory effects on cabbage, carrot, cucumber, onion, pepper, squash and tomato where the roots were generally affected. Those results correlate with the results noted in our study where roots are more sensitive than shoots. Dhole *et al*. (2013) noticed that seed germination and seedling growth of maize were inhibited when using aqueous extracts from the root, stems and leaves of *A*. *tricolor*. Literature and our results indicate that concentration plays a major role in the severity of allelopathic effects on different plants (Qasem, 1995; Obaid & Qasem, 2005).

Table 4.12: Radicle length of different vegetable seeds exposed to increasing concentrations of MeOH-H_2O and DCM leaf extracts of *A. cruentus* grown at optimal, cold and hot temperatures.

Extract [mg mL^{-1}]	Radicle length of Cucumber (mm)			
MeOH-H_2O	**Temperature Treatment**			
	Optimal	**Cold**	**Hot**	**AVG**
0	24±18.45	24±18.45	24±18.45	**24 a**
0.5	4.46±2.22	6.08±3.09	13.54±4.94	**8.03 b**
2	3.46±1.85	2.92±1.49	8.07±3.64	**4.82 b**
AVG	10.64	11	15.20	
$LSD_{(T\leq0.05)}$	**T=ns**	**C=5.21**	**T×C=ns**	
DCM				
0	23.54±14.22	29.92±15.72	23.54±14.22	**25.67 a**
0.5	16.62±5.61	26.62±23.90	16.23±18.85	**19.82 a**
2	26.23±21.92	13.69±16.96	13.92±13.36	**17.95 a**
AVG	22.13	23.41	17.89	
$LSD_{(T\leq0.05)}$	**T=ns**	**C=ns**	**T×C=ns**	

Extract [mg mL^{-1}]	Radicle length of lettuce (mm)			
MeOH-H_2O	**Temperature treatment**			
	Optimal	**Cold**	**Hot**	**AVG**
0	26.31±11.27	26.31±11.27	26.31±11.27	**26.31 a**
0.5	17.54±12.54	13.23±7.25	15.08±6.45	**15.28 b**
2	8±7.43	3.15±3.31	3.85±2.44	**5 c**
AVG	17.28	14.23	15.08	
$LSD_{(T\leq0.05)}$	**T=ns**	**C=4.39**	**T×C=ns**	
DCM				
0	24.77±12.40	28.62±12.10	27.62±11.85	**27 a**
0.5	19.62±5.04	15.77±9.49	8±4.16	**14.46 b**
2	14.08±5.99	9.08±8.84	16.69±6.71	**13.28 b**
AVG	19.49	17.82	17.44	
$LSD_{(T\leq0.05)}$	**T=ns**	**C=4.66**	**T×C=4.66**	

Extract [mg mL^{-1}]	Radicle length of Pepper (mm)			
MeOH-H_2O	**Temperature Treatment**			
	Optimal	**Cold**	**Hot**	**AVG**
0	13.92±5.02	13.92±5.02	13.92±5.02	**13.92 a**
0.5	1.15±2.54	3.31±3.28	9.85±8.71	**4.77 b**
2	0.46±0.88	1.08±0.76	3.46±4.74	**1.67 c**
AVG	5.18	6.10	9.08	
$LSD_{(T\leq0.05)}$	**T=2.29**	**C=2.29**	**T×C=2.29**	
DCM				
0	24.38±11.49	24.38±11.49	24.38±11.49	**24.38 a**
0.5	12.77±3.68	2.85±3.74	1.92±0.86	**5.85 b**
2	5±5.74	6.46±8.90	5.85±6.01	**5.77 b**
AVG	14.05	11.23	10.72	
$LSD_{(T\leq0.05)}$	**T=ns**	**C=3.87**	**T×C=3.87**	

Extract [mg mL^{-1}]	Radicle length of Tomato (mm)			
MeOH-H_2O	**Temperature Treatment**			
	Optimal	**Cold**	**Hot**	**AVG**
0	14.69±6.64	14.69±6.64	14.69±6.64	**14.69 a**
0.5	14.15±4.38	6.92±2.43	15±4.81	**12.02 a**
2	3.69±2.36	10.69±8.51	7.77±5.33	**7.38 b**
AVG	10.84	10.77	12.49	
$LSD_{(T\leq0.05)}$	**T=ns**	**C=2.97**	**T×C=2.97**	
DCM				
0	27.85±13.09	27.85±13.09	27.85±13.09	**27.85 a**
0.5	14.54±13.41	9.54±5.79	10.62±6.09	**11.56 b**
2	4.77±4.91	12.08±4.91	6.92±3.38	**7.92 b**
AVG	15.72	16.49	15.13	
$LSD_{(T\leq0.05)}$	**T=ns**	**C=4.67**	**T×C=ns**	

Different letters along the column indicate significant differences at T≤0.05 (Tukey test). Significant differences within each vegetable; N = 13.

Studies on other plants with allelopatic properties using polar and non-polar extracts described the negative effects on germination and organ development e.g. aqueous and methanol extracts of *Melastoma malabathricum* inhibited germination, root and shoot growth in Barnyard grass seed (Faravanim *et al*., 2008), aqueous, hexane, chloroform and methanol extracts of different organs of *Inula viscosa* had diverse effects on radish, lettuce, peganum and thistle. The degree of inhibition was dependent on the organ extract and the plant it was exposed to. (Omezzine *et al*., 2011). Araniti *et al*. (2016), made methanol-water, hexane, chloroform and ethyl acetate extracts from *Artemesia arborescens.* The *in vitro* bioassay of extracts from *A. arborescens* leaf litter showed a strong inhibitory action on both germination and root growth processes of both tested crops and weeds (*Lactuca sativa*, *Raphanus sativus* and *Amaranthus retroflexus*, *Cynodon dactylon*). These finding confirmed the high phytotoxicity of *A. arborescens* plant tissues reported by Araniti *et al*. (2016). It was also observed that the inhibition caused by the extract was dose dependent, and that each plant species indicated different levels of sensitivity.

Looking from a chemical perspective it is clear that both polar and non-polar compounds were responsible for allelopathy and therefore different compounds are involved. The results also shown a clear change in compound composition and concentration in amaranth plants cultivated at different temperatures. Phenolic concentrations decreased in the heat and cold treated plants and therefore could not be the only compounds responsible for allelopathy in Amaranthus. The vegetables showed different responses to either the plant litter or the extracts and this may possibly be explained by the genetic variation between the seeds, seed size, and seed weight.

References

Allemann, I. 2013. (Unpublished). Phytochemical composition and bioactivity of polar and non-polar leaf extract of three Amaranth varieties with their allelopathic effects on germination and growth of lettuce seedlings. Pp 32.

Amini, R., An, M., Pratley, J., Azimi, S. 2009. Allelopathic assessment of annual ryegrass (*Lolium rigidum*): Bioassays. *Allelopathy J*. **24**(1): 67-79.

Amini, R., Ghanepour, S. 2013. Growth and yield of different types of dry bean affected by smooth amaranth (*Amaranthus hybridus* L.) shoot extracts. *Int. J. Agri. Crop Sci*. **5.2**: 115-119.

Amini, R., Movahedpour, F., Ghassemi-Golezani, K., Mohammadi-Nasab. A.D., Zafarani-Moattar, P. 2012. Allelopathic assessment of common amaranth by ECAM. *Int. Res. J. Appl. Basic Sci.* **3**(11): 2268-2272.

Aparna, V., Dileep, K.V., Mandal, P.K., Karthe, P., Sadasivan, C., Haridas, M. 2012. Anti-inflammatory property of n-hexadecanoic acid: structural evidence and kinetic assessment. *Chem*. *Biol*. *Drug Des*. **80**(3): 434-439.

Araniti, F., Gulli, T., Marrelli, M., Statti, G., Gelsomino, A., Abenavoli, M.R. 2016. *Artemesia. Arborescens* L. leaf litter: phytotoxic activity and phytochemical characterization. *Acta*. *Physiol*. *Plant*. **38**(128): 1-12.

Ayoola, G.A., Coker, H.A.B., Adesegun, S.A., Adepoju-Bello, A.A., Obaweya, K., Ezennia, E.C., Atangbayila, T.O. 2008. Phytochemical Screening and Antioxidant Activities of Some Selected Medicinal Plants Used for Malaria Therapy in Southwestern Nigeria. *Trop*. *J*. *Pharm*. *Res*. **7**(3): 1019-1024.

Bais, H.P., Vepachedu, R., Gilroy, S., Callaway, R.M., Vivanco, J.M. 2003. Allelopathy and exotic plant invasion: from molecules and genes to species interations. *Sci*. **301**: 1377-1380.

Bart, H.J. 2011. Extraction of Natural Products from Plants- An Introduction. Industrial Scale Natural Products Extraction, First Edition. Bart, H.J., Pilz, S (ed). Wiley-VCH Verlag Gmbh and Co. Pp 1-25.

Bertin, C., Yang, X., Weston, L.A. 2003. The role of root exudates and allelochemicals in the rhizosphere. *Plant Soil*. **256**: 67-83.

Bhattacharya, A., Bhaumik, A., Rani, P.U., Mandal, S., Epid, T.T. 2010. Nano-particles – A recent approach to insect pest control. *Afri. J. Biotechnol*. **9**(24): 3489-3493.

Bonanomi, G., Sicurezza, M.G., Caporaso, S., Esposito, A., Mazzoleni, S. 2006. Phytotoxicity dynamics of decaying plant materials. *New Phytol*. **169**: 571–578.

Bondet, V., Brand-Williams, W., Berset, C. 1997. Kinetics and mechanisms of antioxidant activity using the $DPPH^+$ free radicle method. Leben-smittel-Wissenschaft und- Technologie/*Food Sci*. *Tech*. **30**: 609-615.

Broekaert, W.F, Marien, W., Terras F.D., De Bolle M.F., Proost P., Van Damme J., Dillen L., Claeys M., Ress S.B., Vanderleyden J. 1992. Antimicrobial peptides from *Amaranthus caudatus* seeds with sequence homology to the cysteine/glycine-rich domain of chitin-binding proteins. *Biochem*. **31**: 4308-4314.

Carretero, M.E., López-Pérez, J.L., Abad, M.J., Bermejo, P., Tillet, S., Israel, A., Noguera-P, B. 2008. Preliminary study of the anti-inflammatory activity of hexane extract and fractions from *Bursera simaruba* (Linneo) Sarg. (Burseraceae) leaves. *J Ethnopharmaco*. **116**: 11.

Dhole, J.A., Lone, K.D., Dhole, G.A., Bodke, S.S. 2013. Allelopathic effect of aqueous and ethanolic extracts of some common weeds on seed health of *Triticum aestivum* L. (Wheat). *Int*. *J*. *Curr. Mircobiol*. *Appl*. *Sci*. **2**(6): 254-260.

Einhellig, F. A., Eckrich, P. C. 1984. Interactions of tem- perature and ferulic acid stress on grain sorghum and soybeans. *J. Chem. Ecol*. **10**: 161-170.

Faravanim, Baki, H.B., Khalijah, A. 2008. Assessment of allelopathic potential of *Melastomamalabathricum* L. on *Radish raphanussativus* L. and Barnyard Grass (*Echinochloacrus-galli*). *NotulaeBotanicaeHortiAgrobotanici Cluj-Napoca*. **36**(2): 54-60.

FDA. *"Health claims: plant sterol/stanol esters and risk of coronary heart disease (CHD)"*

Fisher, R.F. 1987. Allelopathy: a potential cause of forest regeneration failure. Waller, G.R (ed). Allelochemicals: Role in Agriculture and Forestry, American Chemical Society, Washington, DC (1987). Pp 176-184.

Gamel, T.H., Mesallam, A.S., Damir, A.A., Shekib, L.A.. Linssen, J.P. 2007. Characterization of Amaranth Seed Oils. *J. Food Lipids*. **14**: 323-334.

Guillen, M.D., Manzanos, M.I. 1998. Study of composition of different parts of Spanish *Thymus vulgaris* L. plant. *Food Chem*. **3**: 373-383.

Gunstone, F. D., Harwood, L.J., Dijkstra, A.J. 2007. The Lipid Handbook with Cd-Rom. 3rd ed. Boca Raton: CRC Press, 2007. ISBN 0849396883 | ISBN 978-0849396885.

Guo, P., Al-Khatib, K. 2003. Temperature effects on germination and growth of redroot pigweed (*Amaranthus retroflexus*), Palmer amaranth (*A. plameri*), and common waterhemp (*A. rudis*). *Weed Sci*. **51**: 869-875.

Gupta, A.S., Heinen, J.L., Holaday, A.S., Burke, J.J., Allen, A.D. 2006. Increased resisitance to oxidative stress in transgenic plants that overexpress chloroplastic Cu/Zn superoxide dismutase. PNAS. **90**(4): 1629-1633.

Hart, M.C., Heyl, F.W. 1932. *"Spinasterol and Some of Its Esters". J. Biol. Chem*. **95**(1): 311–5.

Hopkins, W.G., Hüner, N.P.A. 2009. Introduction to Plant Physiology. Chapter 15: Adaptations to the Environment. John Wiley & Son, Inc. USA. Pp 263-274.

Horak, M.J., Loughin, T.M. 2000. Growth analysis of four *Amaranthus* species. *Weed Sci*. **48**: 347-355.

Houghton, P., Raman, A. 1998. Laboratory Handbook for the Fractionation of Natural Extracts. Life Sciences: Biochemistry and Biophysics. ISBN 978-1-4615-5809-5.

Hunter, K.J., Fletcher, J.M. 2002. The antioxidant activity and composition of fresh, frozen, jarred and canned vegetables. *Innov*. *Food Sci*. *Emerg*. *Technolo*. **3**: 399-109.

Inderjit, D. K. M. M. 1994. Allelopathic effect of *Pluchea lanceolata* (Asteraceae) on characteristics of four soils and tomato and mustard growth. *Am. J. Bot*. **81**: 799-804.

Inderjit, D. K. M. M., Einhellig, F. A., eds. 1995. *Allelopathy: Organisms, Processes, and Applications*. ACS Symposium series American Chemical Society, Washington, DC.

James, M.J., Gibson, R.A., Cleland, L.G. 2000. Dietary polyunsaturated fatty acids and anti-inflammatory mediator production. *Am*. *J*. *Clini*. *Nutr*. **71**: 3435-3485.

Jefferson, L.V., Pennacchio, M. 2003. Allelopathic effects of foliage extracts from four Chenopodiaceae species on seed germination. *J*. *Arid Environ*. **55**(2): 275-285.

Jork, H., Funk, W., Fishcer, W., Wimmer, H. 1990. TLC Reagents & Detection Methods- Physical & Chemical Detection Methods: Fundamentals, Reagents Vol 1. Wiley Publishers, New York. Pp 497.

Kalinova, J., Dadakova, E. 2009. Rutin and total quercetin content in amaranth (Amaranthus spp.). *Plant Foods Hum*. *Nutr*. **64**: 68–74.

Kelly, G.S. 1999. Squalene and it potential clinical uses. *Alternative Medicine Review : a Journal of Clinical Therapeutic*. **4**(1): 29-36.

Khan, A.L., Hamayun, M., Hussain, J., Khan, H., Gilani, S.A., Kikuchi, A., Watanabe, K.N., Jung, E.H., Lee, I. 2009. Assessment of allelopathic potential of selected medicinal plants of Pakistan. *Afri. J. Biotechnol*. **8**(6): 1024-1029.

Koeppe, P. E., Rohrbaugh, L.M., Wender, S.H. 1969. The effect of varying UV intensities on the concentration of scopolin and caffeoylquinic acids in tobacco and sunflower. *Phytochem*. **8**: 889-896.

Konovalova, O., Gergel, E., Herhel, V. 2013. GC-MS Analysis of Bioactive Components of Shepherdia Argentea (Pursh.) Nutt. from Ukrainian Flora. *Pharma Inn*. **2** (6): 17.

Konstantinović, B., Blagojević, M., Konstantinivić, B., Samardžić,N. 2014. Allelopathic effect of weed species *Amaranthus retroflexus* L. on maize seed germination. *Rom*. *Agri*. *Res*. **31**: 315-321.

Kothari, V., Seshadri, S. 2010. In vitro antibacterial activity in seed extracts of *Manikara zapota*, *Anona squamosal*, and *Tamarindus indica*. *Biol. Res.* **43**: 165-168.

Kraujalis, P., Venskutonis, P.R., Kraujaliene. V., Pukalskas, A. 2013. Antioxidant Properties and Preliminary Evaluation of Phytochemical Composition of Different Anatomical Parts of Amaranth. *Plant Foods Hum*. *Nutr*. **68**: 322-328.

Król, A., Amarowicz, R., Weidner, S. 2014. Changes in the composition of phenolic compounds and antioxidant properties of grapevine roots and leaves (*Vitis vinifera* L.) under continuous of long-term drought stress. *Acta*. *Physiol*. *Plant*. **36**: 1491–1499.

Lawrey, J.D. 1995. The chemical ecology of lichen mycoparasites: a review. *Canadian J*. *Bot*. **73**(1): S603-S608.

Ling, W.H., Jones, P.J.H. 1995. Minireview dietary phytosterols: A review of metabolism, benefits and side effects. *Life Sci*. **57**(3): 195-206.

Loaiza-Velarde, J.G., Tomás-Barberá, F.A., Saltveit, M.E. 1997. Effect of Intensity and Duration of Heat-shock Treatments on Wound-induced Phenolic Metabolism in Iceberg Lettuce. *J. Amer. Soc. Hort. Sci*. **122**(6): 873-877.

Lü, J.M., Lin, P.H., Yao, Q., Chen, C. 2010. Chemical and molecular mechanisms of antioxidants: experimental approaches and model systems. *J*. *Cell Mol*. *Med*. **14**: 840-860.

Maiyo, Z.C., Ngure, R.M., Matasyoh, J.C., Chepkorir, R. 2010. Phytochemical constituents and antimicrobial activity of leaf extracts of three *Amaranthus* plant species. *Afri. J. Biotechnol.* **9**(21): 3178-3182.

Manikandan, V., Prabhakaran, J. 2014. Allelopathic Influence of Some Weed Residues on Growth and Developmental Changes of Green Gram (*Vigna Radiata (L.) Wilczek*). *Int*. *J*. *Curr*. *Biotechnolo*. **2**(3): 6-10.

McGaw, L.J., Jagar, A.K., Van-Staden, J. 2009. Isolation of antibacterial fatty acids from *Schotia brachypetala*. *Fitoterapia*. **73**: 431-433.

Mendiola, J.A., Santoyo, S., Cifuentes, A., Reglero, G., Ibáñez, E., Señoráns, F.J. 2008. Antimicrobial activity of sub- and supercritical CO2 extracts of the green alga Dunaliella salina, *J*. *Food Prot*. **71**: 2138.

Menges, R.M. 1987. Allelopathic Effects of Palmer Amaranth (*Amaranthus palmeri*) and Other Plant Residues in soil. *Weed Sci*. **35**(3): 339-347.

Menges, R.M. 1988. Allelopathic effect of Palmer amaranth (*Amaranthus palmeri*) on seedling growth. *Weed Sci*. **36**: 325-328.

Mensah, J.K., Okoli, R.I., Ohaju-Obodo, J.O., Eifediyi, K. 2008. Phytochemical, nutritional and medical properties of some leafy vegetables consumed by Edo people of Nigeria. *Afri. J. Biotechnol.* **7**(14): 2304-2309.

Morrison, J.I.L., Lawlor, D.W. 1999. Interactions between increasing CO_2 concentration and temperature on plant growth. *Plant Cell Environ*. **22**: 659-82.

Mroczek, A., 2015. Phytochemistry and bioactivity of triterpene saponins from Amaranthaceae family. *Phytochem*. *Rev*. DOI 10.1007/s11101-015-9394-4.

Obaid, K.A., Qasem, J.R. 2005. Allelopathic activity of common weed species on vegetable crops grown in Jordan. Plant Protection Department, Faculty of Agriculture, University of Jordan, Amman, Jordan.

Omezzine, F., Rinez, A., Ladhari, A., Farooq, M., Haouala, R. 2011. Allelopathic potential of *Inula viscosa* against crops and weeds. *Int. J*. *Agric*. *Biol*. **13**: 841-849.

Palic, R., Stojanovic, G., Alagic, S., Nikolic, M., Lepojevic, Z. 2002. Chemical composition and antimicrobial activity of the essential oil and CO2 extracts of the oriental tobacco, Prilep. *Flavour Fragr. J.* **17**: 323–326.

Paśko, P., Sajewicz, M., Gorinstein, S., Zachwieja, Z. 2008. Analysis of selected phenolic acids and flavonoids in Amaranthus cruentus and Chenopodium quinoa seeds and sprouts by HPLC. *Acta*. *Chromatogr*. **20**: 661–672.

Qasem, J.R. 1995. The allelopathic effect of three *Amaranthus* spp. (pigweeds) on wheat (*Triticum durum*). *Weed Res*. **35**(1): 41-49.

Qasem, J.R. 2010. Allelopathy Importance, Field Applicantion and Potential Role in Pest Management: A Review. *J*. *Agri*. *Sci*. *Tech*. **4**(6): 104-120.

Quayyam, J.R., Malik, A.V., Leach, D.M., Gottardo, C. 2000. Growth inhibitory effects on nut grass (*Cyperus rotundus*) on rice (*Oryza sativa*). *J. Chem. Ecol*. **26**: 2221-2231.

Rahimzadeh, F., Tobeh, A., Jamaati-e-Somarin, S. 2012. Study of allelopathic effects of aqueous extracts of roots and seeds of goosefoot, red-root amaranth and field bindweed on germination and growth on lentil seedlings. *Int*. *J. Agron*. *Plant Prod*. **3**(9): 318-326.

Rajasekar, M., Arumugam. T., Kumar, S.R. 2013. Influence of weather and growing environment on vegetable growth and yield. *J*. *Hort*. *Fores*. **5**(10): 160-167.

Reddy, L.H., Couvreur, P. 2009. Squalene: A natural triterpene for use in disease management and therapy. *Elsevier: Advanced Drug Delivery Reviews*. **61**: 1412-1426.

Rice, E.L. 1979. Allelopathy- An Update. *Bot*. *Rev*. **45**(1): 15-109.

Rodriguez-Leyva, D., Bassett, C.M.C., McCullough, R., Pierce, G.N. 2010. The cardiovascular effects of flaxseed and its omega-3 fatty acid, alphalinolenic acid. *Can*. *J. Cardiol*. **26**(9): 489-496.

Rudikovskaya, E.G., Fedorova, G.A., Dudareva, L.V., Makarova, L.E., Rudikovskii, A.V. 2008. Effect of Growth Temperature on the Composition of Phenols in Pea Roots. *Russian J*. *Plant Physiolo.* **55**(5): 712-715.

Rungismakan, S. 2011. Phytochemical and biological activity studies on *Salvia viridis* L. Thesis. University of Bath. Pp 1-413.

Sadaqa, E.A., Bawazir, A.A., Qasem, J.R. 2010. Allelopathic activity of some common weeds species in onion fields. *Allelopathy J*. **26**(2): 175-184.

Shah, A.H.A. 2005. Spinoside, new coumaroyl flavone glycoside from *Amaranthus spinosu*s. *Arch*. *Pharmacal*. *Res*. 27(12):1216-1219.

Sivakumar, R., Dhivya, A. 2015. GC-MS analysis of bioactive compounds on ethyl acetate extract of *Cordia monoica roxb*. leaves. *Int*. *J*. *Res*. *Develop. Pharm. Life Sci*. **4**(1): 1328-1333.

Steffensen, S.K., Rinnan, Å., Mortensen, A.G., Laursen, B., de Troiani, R.M., Noellemeyer, E.J., Janovska, D., Dusek, K., Délano-Frier, J., Taberner, A., Christophersen, C., Fomsgaard, I.S. 2011. Variations in the polyphenol content of seeds of field grown Amaranthus genotypes. *Food Chem*. **129**: 131–138.

Stern, K.R. 2006. Introductory Plant Biology, tenth edition. McGraw Hill. New York. Pp 624.

Svirskis, A. 2003. Investigation of cultivation and utilization in Lithuania. *Agron. Res*. **1**(2): 253-264.

Szakiel, A., Paczkowski, C., Henry, M. 2010. Influence of environmental abiotic factors on the content of saponins in plants. *Phytochem*. *Rev*. **2**: 25.

Tabrizi, E.F.M., Yarnia, M. 2011. Allelopathy extracts various parts of pigweed germination and seedling growth corn. *Ann*. *Biol*. *Res*. **2**(5): 83-86.

TLC stains. 2016. lcso.epfl.ch/files/content/sites/lcso/files/load/TLC_Stains.pdf.

Vidović, M., Morina, F., Milić, S., Jovanović, S.V. 2015. An improved HPLC-DAD method for simultaneously measuring phenolics in the leaves of *Tilia*

platyphyllos and *Ailanthus altissima*. Institute of Botany and Botanical Garden Jevremovac, Belgrade. *Botanica SERBICA*. **39** (2): 177-186.

Wagner, H., Bladt, S. 1996. Plant Drug Analysis: A Thin Layer Chromatography Atlas. 2nd Edition. Springer-Verlag Berlin Heidelberg, Germany. Pp 384.

Wang, C.Y. 1982. Physiology and biochemical response of plant chilling stress. *Hort Sci*. **17**: 173-186.

Wang, S.Y., Zheng, W. 2001. Effect of plant growth temperature on antioxidant capacity in strawberry, *J. Agric. Food Chem*. **49**: 4977–4982.

Wender, S. H. 1970. Effects of some environmental stress factors on certain phenolic compounds in tobacco. *Rec. Adv. Phytochem*. **3**: 1-29.

Wink, M. 1999. Biochemistry of plant secondary metabolism. *Ann*. *Plant Rev*. **2**: 1-374.

Woyengo, T.A., Ramprasath, V.R., Jones, P.J.H. 2009. "Anticancer effects of phytosterols". *Euro*. *J*. *Clin*. *Nutr*. **63**(7): 813-820.

Yamada, H., Kiyohara, H., Nagai, T., Sunazuka, T. 2009. Vaccine preparation containing fatty acid as a constituent. US007560484B2. Pp 1-40.

Zhao, G., Etherton, T.D., Martin, K.R., Vanden Heuvel, J.P., Gillies, P.J., West, S.G., Kris-Etherton, P.M. 2005. Anti-inflammatory effects of polyunsaturated fatty acids in THP-1 cells. *Biochem*. *Biophys*. *Res*. *Commun*. **336**(3): 909-917.

Zheng, L., Wang, L., Guo, X., Li, X., Chen, Z. 2011. Essential oil composition from the seeds of *Dalbergia odorifera* T. Chen grown in Hainan, China. *Food, Agri*. *Environ*. *(JFAE)*. **9**(3&4): 26-28.

CHAPTER 5

GENERAL DISCUSSION AND CONCLUSION

5.1 General discussion and conclusions

Amaranth is an underutilised and under researched plant which has enormous potential as a food source, as herbal medicine and as a natural herbicide. Not many studies have been done on grain amaranth but because this plant species is starting to become established as a new food source it is important to understand not just the competition aspect but also the allelopathic impact on succeeding plant species. Research on the allelopathic aspects of amaranth is cited in literature but the allelopathic aspects of stressed *A. cruentus* is lacking. Climate change is a reality and the impact of climate change on the allelopathic properties is important in the cultivation of crops in rotation practices.

This study was aimed at contributing to the knowledge of the allelopathic potential of the foliage of *A. cruentus* under optimal and temperature stress environments.

5.2 Survival and chemical composition

5.2.1 Optimal temperature

There was a 100% survival rate when plants were exposed to optimal growing conditions. The overall mass of the plants was higher than the heat treated plants but less than the cold treated plants. Total phenolic and flavonoid content were significantly higher than in both the heat and cold treated plants (Table 4.4). Extracts also showed prominent antioxidant activity when treated with DPPH which relate to the amount of phenolic compounds (Fig. 4.3). Ayoola *et al.* (2008), showed that there is a good correlation between flavonoid content and antioxidant activity.

Thin layer chromatography indicated the optimal methanol-water extract contained the most compounds (Fig. 4.2) while the least were detected in the DCM extract (Fig. 4.2). HPLC graphs confirmed that optimum extracts have many more peaks than the other two treatments, with rutin and catechin major components. Phosphine imide, P, P, P-triphenyl was identified as major compound in the DCM extract. These results showed that plants grown under optimal conditions still produce many compounds that can possibly influence other plants.

5.2.2 Heat stress

When plants were exposed to hot treatments, there was only a 72% plant survival rate which indicated that these temperatures were unfavourable with the overall mass of the plants being lower than plants grown at other temperatures. *Amaranthus* species are C4-plants and it is well-known that C4-plants are generally tropical or subtropical, therefore are more resistant to heat (Hopkins & Hüner, 2009). Total phenolic and flavonoid content was significantly lower in the heat treated leaf litter, which may indicate that PAL activity was influenced by the higher temperature (Loaiza-Velarde *et al*., 1997), therefore influencing the biosynthesis of phenolic compounds.

Thin layer chromatography showed that the heat treated methanol-water plant extracts contained less phenolic compounds while a wide variety of other possible compounds including terpenes, saponins and sugars were found (Wagner & Bladt, 1996). Antioxidant activity was detected, although not as prominent as in methanol-water extracts of the other temperature treatments. Kraujalis *et al*. (2013) found that acetone and methanol-water extracts of different plant organs and seeds of amaranth, displayed antioxidant activity. In the methanol-water extract, less compounds were visible on TLC plates which correlates with the HPLC graphs that contained fewer phenolic compound peaks (Fig. 4.5). In the polar extract of the hot treatment none of the phenolic compound standards (gallic acid, cathechin, caffeic acid, rutin and quercetin) were detected by HPLC.

Only the methanol-water heat extract displayed antibacterial activity when exposed to *E. coli*. It may therefore be possible that this antibacterial compound can have an influence on soil bacteria and change the soil rhizosphere.

Thin layer chromatography of the DCM extract indicated that there were more compounds present in the heat treatment than in the other treatments. A prominent blue spot appeared at R_f = 0.83 on the TLC plate which was projected to be a terpene and this compound tested positive for antioxidant properties. Through GC-MS analysis, dichloroacetic acid, tridec-2-ynyl ester was the major compound identified, although there was no information on the bioactivity found in literature.

These findings indicated that plants under heat stress synthesised less phenolic (polar) compounds and also different compounds compared to other temperature stresses. Many authors reported on the association of phenolic compounds with allelopathy (Inderjit *et al*., 1995; Ferguson *et al.,* 2003; Li *et al*., 2010; Trezzi *et al*., 2016). In this study, hot temperatures decreased phenolic compounds, indicating that there are also other compounds that are also responsible for phytotoxicity.

5.2.3 Cold stress

Exposure to cold temperature displayed a 98% survival rate with the overall mass of the plants being higher than plants that were grown at other temperatures. Total phenolic and flavonoid content was significantly lower than the optimal treated plants but not significant when compared to the heat treatment. In a study done by Rudikovskaya *et al*. (2008), on peas grown at lower temperatures a decrease in phenolic content in seedling roots was noted.

Thin layer chromatography showed that the methanol-water extract contained phenolics and other compounds. Cold treatment had more compounds than the heat treatment but less than the optimal treatment (Fig. 4.1) which correlates with the HPLC graphs where rutin, catechin and quercetin were identified (Fig. 4.5 & 4.6). In the DCM extracts, neophytadiene and 9,12,15-octadecatrienoic acid were the major compounds detected by GC-MS. Extracts also showed antioxidant activity when treated with DPPH and Palic *et al*. (2002) reported on the antioxidant activities of neophytadiene.

In general the different temperature stresses both cold and hot, changed the chemical composition of both the polar and non-polar extracts. The total phenolic compounds in the hot treatment were significantly lower when compared to the cold and optimal treatments. The compounds identified in non-polar extracts needs to be purified to proof/determine the compounds involvement in allelopathy.

5.3 Phytotoxicity

5.3.1 Germination percentage

- **Litter**

The *in vitro* bioassay of the plant litter from *A. cruentus* indicated inhibitory action on germination of all four tested vegetable species for all the treatments. Vegetables that were exposed to heat treated amaranth litter showed most severe inhibition in germination of 75% (Fig. 4.13). Peppers were the most sensitive to the heat treated litter, followed by cucumber, tomato and lettuce (Fig. 4.11). Lettuce seeds on the other hand, were most inhibited when exposed to optimal treated litter. The inhibition caused by the litter was dependant on dose and each vegetable species showed different levels of sensitivity. Vegetables can be either planted directly into the soil by using seeds or by transplanting seedlings (Allemann & Young, 2005; Agricultural Research Council, 2013). Although some vegetables (i.e. Sweet pepper) are planted as seedlings (Allemann & Young, 2005) these results added new information to the sensitivity of seed germination. In crop rotation systems it is important, because if germination is affected by compounds *of A. cruentus* produced under stress conditions, farmers can incur financial losses. Even though crop seeds were not tested it is recommended that these tests are also applied to crops.

- **Extracts**

Both polar and non-polar extracts significantly inhibited germination of all vegetables ($LSD_{(T\leq0.05)}$ = 1.88) when exposed to different concentrations of extracts of the different temperature treatments (Table 4.10). At 0.5 mg mL^{-1}, germination of peppers was reduced by 91% when exposed to the methanol-water extract of optimally treated plants. Lettuce also showed highest inhibition when exposed to optimal treatment, while cucumber and tomato were most severely inhibited when exposed to cold and heat polar extracts respectively.

The highest concentrations (2 mg mL^{-1}) of the different DCM extracts were most inhibitory on germination of all vegetables. The optimal extract resulted in best inhibition of cucumber while pepper and lettuce were more affected by the cold extract (Table 4.10). No significant inhibition in tomato seeds germination was

noticed between the different temperature treatments of DCM extracts. When comparing the vegetables, peppers germination was the most reduced.

There were also differences in the potential to inhibit germination between the solvent extracts, showing that different compounds can play a role in the reaction of each vegetable. Some vegetables may be more sensitive to polar compounds where others may be more damaged by non-polar compounds.

All the extracts of all the temperature treatments had an influence on the germination of all tested vegetables and this includes the optimal (stress treatment control) temperature.

5.3.2 Hypocotyl length

- **Litter**

Cucumber and peppers only showed significant reduction in organ length at 1 mg mL^{-1}, only tomato seeds show significant inhibition at both concentrations (Fig. 4.14). Although tomato and pepper seeds were not affected by the temperature treatment, they were affected by an increase in concentration of the litter which significantly inhibited hypocotyl length. Cucumber seeds were influenced by the temperature treatments and indicated that the heat treatment inhibited length the most (Fig. 4.15). Lettuce showed a two way interaction with both concentration and temperature treatment (Fig. 4.16). The heat treatment had the biggest effect on the growth of lettuce hypocotyl with a reduction of 92% at 5 mg mL^{-1}.

- **Extracts**

Both polar and non-polar extracts significantly reduced the hypocotyl length of all vegetables at 2 mg mL^{-1} (Table 4.11). Methanol-water extracts caused the most severe reduction in hypocotyl lengths of cucumber and pepper at 2 mg mL^{-1}, while DCM showed this trend in tomatoes only. Only the DCM extracts of the cold and hot treatments played a significant role.

5.3.3 Radicle length

- **Litter**

Radicle (root) growth of cucumber, pepper and tomato were significantly influenced by the concentration of the temperature treated plant litter (Fig. 4.17). Tomato showed no significant inhibition of radicle development with increased litter concentrations. Concentration and temperature treatments were highly significant in reducing radicle length of lettuce seedlings with optimal treatment having the highest effect (Fig. 4.19).

- **Extracts**

Both polar and non-polar extracts significantly reduced the radicle length of all vegetables at 2 mg mL^{-1} (Table 4.12). Methanol-water extracts caused the most severe reduction in radicle lengths of cucumber, lettuce and pepper at 2 mg mL^{-1}, while DCM showed this trend in peppers only. The methanol-water extracts of the optimal temperature treatment played a significant role in only pepper.

All the vegetables that were tested reacted differently towards the different temperature treatments. Both the hypocotyl and the radicle of all the tested vegetables showed inhibition when exposed to all the temperature treatments; however the heat stress treatment played the largest role. Another aspect that played a significant role was the concentration of the litter and extracts. Increasing concentrations caused more damage to the growth of most vegetables. Consequently, if more plant residues (leaf litter) are left behind on the land, the growth of the next crop will often be affected with a subsequent decline in yield.

In conclusion, temperature influenced the chemical composition of *A. cruentus* and *in vitro* bioassays proved the negative impact of litter and extracts on germination and growth of vegetables. This demonstrated that the environment for the cultivation of *A. cruentus* is important and that more than one compound were responsible for allelopathy, thus both polar and non-polar compounds were involved. Furthermore, with increased concentrations of both litter and extracts a decrease in germination and seedling development occurred. Therefore, it is possible that more leftover plant material in a field can have a more severe allelopathic effect on the following

harvest. It was also clear that vegetables displayed diversity in reaction towards the temperature treatments and type of extract.

According to literature, this is the first report on the *in vitro* phytotoxicity of a DCM extract of grain amaranth; therefore the results are important in showing that non-polar compounds present in *A. cruentus* might play a significant role in allelopathy.

Future studies in soil are vital to determine if the *in vitro* results can be repeated in field studies. This will add to the understanding of allelopathic properties of *A. cruentus* in crop rotating systems. Purification and identification of active compounds needs to be performed in order to find novel natural herbicides.

References

Agricultural Research Council. 2013. Production guidelines for summer vegetables. ARC-Vegetable and Ornamental Plant Institute (ARC-VOPI). Pp 1-44.

Allemann, L., Young, B.W. 2015. Vegetable production in a nutshell. Kwa-Zulu Natal Department of Agriculture. Pp 1-34.

Ayoola, G.A., Coker, H.A.B., Adesegun, S.A., Adepoju-Bello, A.A., Obaweya, K., Ezennia, E.C., Atangbayila, T.O. 2008. Phytochemical Screening and Antioxidant Activities of Some Selected Medicinal Plants Used for Malaria Therapy in Southwestern Nigeria. *Trop*. *J*. *Pharm*. *Res*. **7**(3): 1019-1024.

Ferguson, J.J., Rathinasabapathi, B., Chase, C.A. 2003. Allelopathy: How Plants Suppress Other Plants. University of Florida. IFAS Extension. Pp 5.

Hopkins, W.G., Hüner, N.P.A. 2009. Introduction to Plant Physiology. Chapter 15: Adaptations to the Environment. John Wiley & Son, Inc. USA. Pp 263-274.

Inderjit, D., K. M. M., Einhellig, F. A., eds. 1995. *Allelopathy: Organisms, Processes, and Applications*. ACS Symposium series American Chemical Society, Washington, DC.

Kraujalis, P., Venskutonis, P.R., Kraujaliene. V., Pukalskas, A. 2013. Antioxidant Properties and Preliminary Evaluation of Phytochemical Composition of Different Anatomical Parts of Amaranth. *Plants Food Hum*. *Nutr*. **68**: 322-328.

Li, Z.H., Wang, Q., Ruan, X., Pan, C.D., Jiang, D.A. 2010. Phenolics and Plant Allelopathy. *Molecules*. **15**: 8935-8952.

Loaiza-Velarde, J.G., Tomás-Barberá, F.A., Saltveit, M.E. 1997. Effect of Intensity and Duration of Heat-shock Treatments on Wound-induced Phenolic Metabolism in Iceberg Lettuce. *J. Amer. Soc. Hort. Sci*. **122**(6): 873-877.

Palic, R., Stojanovic, G., Alagic, S., Nikolic, M., Lepojevic, Z. 2002. Chemical composition and antimicrobial activity of the essential oil and CO2 extracts of the oriental tobacco, Prilep. *Flavour Fragr. J.* **17**: 323–326.

Paśko P., Sajewicz M., Gorinstein S., Zachwieja Z. 2008. Analysis of selected phenolic acids and flavonoids in Amaranthus cruentus and Chenopodium quinoa seeds and sprouts by HPLC. *Acta Chromatogr*. **20**: 661–672.

Ruikovskaya, E.G., Fedorova, G.A., Dudareva, L.V., Makarova, L.E., Rudikovskii, A.V. 2008. Effect of Growth Temperature on the Composition of Phenols in Pea Roots. *Russian J*. *Plant Physiol.* **55**(5): 712-715.

Trezzi, M.M., Vidal, R.A., Balbinot Junior, A.A., Bittencourt, H.H., da Silva Souza Filho, A.P. 2016. Allelopathy: driving mechanisms governing its activity in agriculture. *J*. *Plant Inter*. **11**(1): 53-60.

Wagner, H., Bladt, S. 1996. Plant Drug Analysis: A Thin Layer Chromatography Atlas. 2nd Edition. Springer-Verlag Berlin Heidelberg, Germany. Pp 384.

Summary

Allelopathic plants disrupt germination and growth of agricultural crops which will lead to a lower yield production. However, allelopathy is also important in an environmentally friendly approach to control weeds and reduce chemical herbicide usage. Allelochemicals are secondary metabolites, released into the environment through leaching, volatile emissions, root exudation and the decaying of plant residues. The isolation and identification of allelochemical compounds involved in the plant-plant interactions are of great importance.

Amaranthus cruentus is used for both its grain and leaves for consumption. This plant is now being considered at as a new agricultural crop; therefore it is important to understand how this plant will interact within a changing environment and how this will influence other plants. It is known that amaranth plants have the ability to synthesize allelochemicals in order to protect itself from different types of stress. In this study, the leaf litter as well as the polar and non-polar extracts of the leaves of *A. cruentus* from three different temperature regimes were investigated for their *in vitro* allelopathic properties towards tomato, pepper, cucumber and lettuce. The chemical compounds induced during each temperature treatment were also compared.

Methanol-water and dichloromethane (DCM) crude extracts were prepared from each of the temperature treated amaranth plants using dried ground leaf litter. The identification of compounds present in the different treated plants was done through thin layer chromatography (TLC), high pressure liquid chromatography (HPLC) and gas chromatography coupled to mass spectrometry (GC-MS). TLC indicated the presence of phenolics, terpenes and flavonoids in all *A. cruentus* extracts. Differences were seen in the number and type of compounds between the temperature treated plant extracts.

Methanol-water extracts of the three plant treatments were analysed using HPLC with specific consideration to phenolic compounds. The optimal treatment showed the most peaks followed by the cold and lastly the heat treatment that showed the least amount of peaks. Compounds identified in the optimal and cold treatments included catechin and rutin, while the cold treatment also contained quercetin. The heat treatment contained none of the compounds that were used as standards.

Dichloromethane (DCM) extracts of the three plant treatments were analysed using GC-MS. In the optimal treatment, the compound found in the highest abundance was phosphine imide, P, P, P-triphenyl (38.59%), the cold treatment contained neophytadiene (27.35%) and α-linolenic acid (24.41%). Dichloroacetic acid (29.68%) was identified as the major compound in the DCM extract of *A. cruentus* heat treated plant litter.

Laboratory bioassays were carried out to evaluate the *in vitro* allelopathic activity of different concentrations of the stressed amaranth plant litter or methanol-water and DCM crude extracts, against four vegetables (tomato, lettuce, cucumber and pepper) by means of the Sandwich Method.

Germination, hypocotyl and radicle length were adversely influenced by the plant litter for all the temperature treatments. The inhibition caused by the litter was dependant on concentration and each vegetable species showed different levels of sensitivity.

The extracts indicated that both types of extracts and all temperature treatments had a negative influence on the germination of all the vegetables. The methanol-water and DCM extracts adversely affected organ length for all the vegetables at the highest concentration for each of the treatments. The severity of inhibition was dependent on both the concentration and temperature treatment. Each vegetable species showed different levels of sensitivity against the different extract treatments.

The results obtained in this study, demonstrated that cold, hot and optimal temperature treatments induced the expression of different chemical compounds in the leaves of *A. cruentus* plants. All temperature treatments however affected germination and growth of some vegetables, exhibiting allelopathic properties. Therefore, plant litter and both polar and non-polar extracts of all the treated amaranth plants, contained compounds responsible for allelopathy. Furthermore, the results indicated that vegetables reacted differently towards the treatments of the leaf litter and extracts, showing variation in sensitivity against various compounds.

Thus, abiotic stress does have an influence on both the chemical composition and allelopathy of *A. cruentus*, but it was likewise proven that plants grown under optimal

growth conditions also produced both polar and non-polar compounds with allelopathic properties which may have a negative impact in crop rotation systems.

Keywords: allelopathy, phenolic compounds, extracts, *A. cruentus*, vegetables, germination, hypocotyl, radicle.

Opsomming

Allelopatiese plante ontwrig ontkieming en groei van landbougewasse wat kan lei tot laer opbrengste. Allelopatie is egter ook belangrik in 'n omgewingsvriendelike benadering vir onkruidbeheer en om die gebruik van chemiese onkruiddoders te verminder. Allelochemikalieë is sekondêre metaboliete, wat in die omgewing vrygestel word deur loging, vlugtige vrystellings, wortel afskeiding en die ontbinding van plantmateriaal. Die isolasie en identifisering van allelochemiese verbindings wat betrokke is by plant-plant-interaksies is daarom van groot belang.

Beide die graan en blare van *Amaranthus cruentus* word gebruik. Hierdie plant word nou oorweeg as 'n nuwe landbougewas, daarom is dit belangrik om te verstaan hoe hierdie plant binne 'n veranderende omgewing sal reageer en hoe dit ander plante kan beïnvloed. Amarantus plante is daarvoor bekend dat hulle die vermoë het om allelochemikalieë te sintetiseer vir beskerming teen verskillende tipes stres situasies. In hierdie studie was *A. cruentus* onder drie verskillende temperatuur toestande gekweek en die *in vitro* allelopatiese eienskappe van die droë blaarmateriaal (residu), asook polêre en nie-polêre ekstrakte daarvan, teenoor tamatie, peper, komkommer en blaarslaai getoets. Die chemiese verbindings wat gedurende die verskillende temperatuur toestande gesintetiseer is, was ook vergelyk.

Metanol-water en dichlorometaan (DCM) ekstrakte was berei uit die residu van die verskillende temperatuurstres behandelde amarantus plante. Identifisering van die verbindings in die verskillende plante is gedoen deur middel van dunlaagchromatografie, hoë druk vloeistofchromatografie en gaschromatografie gekoppel aan massaspektrometrie. Dunlaagchromatografie het die teenwoordigheid van fenole, terpene en flavonoïede in alle *A. cruentus* ekstrakte aangedui. Verskille in die aantal verbindings tussen die verskillende temperatuur- behandelde ekstrakte is opgemerk.

Hoë druk vloeistofchromatografie, met spesifieke verwysing na fenoliese verbindings, is gebruik om die metanol-water ekstrakte van die drie temperatuur-behandelings te ontleed. Die optimale temperatuurbehandeling het die meeste pieke getoon, gevolg deur die koue- en laastens die hittebehandeling wat die minste pieke

getoon het. Verbindings in die optimale- en kouebehandelings het catechin en rutin ingesluit, terwyl die kouebehandeling ook quercetin bevat het. Die hittebehandeling het geen van die verbindings wat as standaarde gebruik is, bevat nie.

Die dichlorometaan (DCM) ekstrakte van die drie temperatuurbehandelings is ontleed met behulp van gaschromatografie gekoppel aan massaspektrometrie. In die optimalebehandeling was die verbinding met die hoogste voorkoms fosfien imide, P, P, P-triphenyl (38,59%), terwyl die kouebehandeling neophytadiene (27,35%) en α-linoleensuur (24,41%) bevat het. Dichloroasynsuur (29,68%) is geïdentifiseer as die belangrikste verbinding in die DCM ekstrak van die hitte-behandelde *A. cruentus* plantmateriaal.

Laboratorium analises is uitgevoer om die *in vitro* allelopatiese aktiwiteit van verskillende konsentrasies van die amarantus plantresidu en ekstrakte (metanol-water en DCM) teen vier groente (tamatie, blaarslaai, komkommer en peper) te evalueer deur gebruik te maak van die “Sandwich” metode.

Ontkieming, hipokotiel en kiemwortel lengte was ongunstig beïnvloed deur die plant residu vir al die behandelings. Die remming wat deur die residu veroorsaak is, is afhanklik van die konsentrasie en elke spesie groente het verskillende vlakke van sensitiwiteit getoon.

Die ekstrakte dui daarop dat beide tipes ekstrakte en al die temperatuur-behandelings 'n negatiewe invloed het op die ontkieming van al die groente. Die hoogste konsentrasies van die metanol-water en DCM ekstrakte vir elk van die behandelings, veroorsaak 'n afname in orgaan lengte in al die groente. Die mate van inhibisie was afhanklik van beide die konsentrasie en temperatuur-behandeling. Soos vir die plant residu, toon elke groente spesie verskillende vlakke van sensitiwiteit teenoor die verskillende ekstrakbehandelings.

Die uitslag van hierdie studie, het getoon dat koue, warm en optimale temperatuur behandelings gelei het tot die induksie van verskillende chemiese verbindings in die blare van *A. cruentus* plante. Al die temperatuur behandelings het egter ’n negatiewe invloed op ontkieming en groei van sekere groente gehad, wat op allelopatie dui. Dus, bevat die plant residu en beide polêre en nie-polêre ekstrakte van al die behandelde amarant plante, verbindings wat verantwoordelik is vir allelopatie.

Verder toon die resultate dat groente verskillend reageer op die verskillende behandelings, wat dui op ’n variasie in sensitiwiteit teenoor verskillende verbindings.

Abiotiese stres het dus wel 'n invloed op die chemiese samestelling en allelopatiese eienskappe van *A. cruentus*, alhoewel dit ook bewys is dat plante wat onder optimale groeitoestande gekweek is, beide polêre en nie-polêre verbindings bevat wat allelopatie veroorsaak en 'n negatiewe impak op wisselboustelsels kan hê.

Sleutelwoorde: allelopatie, fenoliese verbindings, ekstrakte, *A. cruentus*, groente, ontkieming, hipokotiel, kiemwortel.