Harper Adams University

A Thesis Submitted for the Degree of Doctor of Philosophy at

Harper Adams University

Harper Adams University

Weed control using allelopathic plant species

By

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(BSc. & MSc.)

Thesis submitted in partial fulfilment to the requirements for the award of the degree of Doctor of Philosophy by Harper Adams University.

June 2016

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Declaration

I declare that the work presented in this thesis is an original compilation by the author. None of this work has been presented in any previous application for any degree or qualification. All the relevant sources of information referred to in this thesis are cited within the text and details presented in the references section.

Signed……..

Hadi Moso Ali Aliki

Abstract

Several laboratory experiments were conducted during the course of this project to test the allelopathic effects of *Brassica napus* L. leaves, stems, roots and flowers on three weed species *Phalaris minor* (Retz.), *Convolvulus arvensis* (L.) and *Sorghum halepanses* (L.) germination and growth, and to determine the glucosinolates profile and their concentration in *Brassica napus* tissues.

In this study, it was found that all water extract treatments from different *Brassica napus* parts and under different concentrations had the ability to inhibit weed species germination and growth significantly. Exposure to flower and stem extracts caused the greatest reduction in the seed germination and seedling growth of all weed species that were tested in this study.

Water extracts from different *Brassica napus* parts and during different plant development stages significantly inhibited the seed germination and growth of all weed species. Glucosinolates profiles and concentrations in *Brassica napus* tissues were significantly different between different plant parts during different plant development stages. Progoitrin was the dominant glucosinolate in *B. napus* flowers and gluconasturtiin in roots. However, flower extracts were more effective in weed management as compared with root extracts.

Applying aqueous solution of pure glucosinolate significantly inhibit seed germination and seedling growth. Glucosinolate types and their concentrations linked positively with weed species inhibition.

Water extract from different parts of *B. napus* during water stress conditions under all plant development stages demonstrated variability in their effect on germination and growth of weed species between the water stress levels and within the same plant development stage. Furthermore, glucosinolates concentrations and

myrosinase activity in *B. napus* tissues were significantly different between different plant parts during the water stress conditions and under different plant development stages. This project has revealed that using water extracts from *B. napus* may play an important role in weed species inhibition.

Acknowledgement

I would like to express my gratefulness to several people that helped this thesis being made. First of all, I would like to thank my supervisors, Dr John Reade, who has supported me with continuous guidance, support and encouragement. Many thanks to my second supervisors Dr Matthew Back for his assistance, guidance and support throughout this research project.

Many thanks go to Professors Peter Kettlewell, Dr. Andrew Wilcox, Dr. Simon Edwards and Dr. Ivan Grove for their support and advices in turn of statistical analysis.

I would like to thank all the staff of Crop and Environment Research Centre (CERC) specially Mrs. Jan Haycox for her help in the glasshouse.

Many thanks go to the staff of Princess Margaret and Engineering Laboratories especially Dr. Victoria Talbot, Amjad Ali, Kevin Jones, Thomas Leigh for their kind support. Special thanks also go to Jo Rawlinson and Sarah Estibeiro for their support.

I am very grateful for the friendship of all of the PhD students, especially. Tijana Stancic.

I express my sincere sense and heartiest gratitude to my parents and my family and, Widad, Dima, Sara and Adam for their support and encouragement during the course of this project.

STATEMENT OF CONTRIBUTIONS TO PUBLICATIONS

Research articles:

Published

HADI. M. ALIKI, JOHN .P.H. READE and MATTHEW A. BACK. 2014. Effects of concentrations of *Brassica napus* (L.) water extracts on the germination and growth of weed species. *Allelopathy Journal,* 34 (2), pp. 287-298

Conference presentations

Oral presentation at:

- Presentation Skills Training held at Harper Adams University, 19 April 2013.
- Annual postgraduate colloquium held at Harper Adams University, 26 November 2012.
- Annual postgraduate colloquium held at Harper Adams University, 28 November 2013.
- Lunch time Research seminar held at Harper Adams University, Jun 2013.
- The 7th World Congress on Allelopathy Complex Interactions in a Changing Climate, July 28 - August 1, 2014, Vigo, Spain.
- The 5th International Symposium of Biofumigation, 9 – 12 September 2014, Harper Adams University, Newport, Shropshire.

Poster:

- Annual postgraduate colloquium held at Harper Adams University, 24 November 2013.
- The 50th Annual BCPC Weed Review, 14 November 2013, Rothamsted Research, Harpenden, Herts, UK.
- The 7th World Congress on Allelopathy Complex Interactions in a Changing Climate, July 28 - August 1, 2014, Vigo, Spain,
- The UK PlantSci, Uk Plant sciences federation, 14-15 April 2015, Harper Adams University, Newport, Shropshire.

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Chapter 1

1. Review of Literature

1.1. Introduction

Weeds are plants that are adapted to a wide range of environmental conditions and interfere with crops (Cobb and Reade, 2010). They compete with cultivated crops for nutrients, moisture, sunlight and space, as well as harbouring pests and diseases that damage crops and reduce yields (Singh *et al*., 2001). Weeds are major problems in crop production worldwide. Weeds are mainly combated through the use of herbicides and manual and mechanical control methods such as weed pulling, mowing, mulching, tilling, soil solarization and flooding (Tu *et al.,* 2001). However, since all of these methods may have adverse impacts on agro eco-systems alternative strategies are required (Lawley, 2010).

In recent times in developing countries, herbicides have been favoured by farmers, but they face many problems in inefficient weed control because of a lack of information in herbicide technology. Poor application of herbicides can lead to serious ecological and environmental problems such as increased herbicide resistance weeds, groundwater contamination and pollution (Jamil, 2004). Moreover, because of a lack of awareness and unsuitable regulatory and preventive mechanisms, the users and consumers in developing countries are facing pesticide-related health problems (Gupta *et al*, 2008). In general, using chemical weed control is unsafe and may increase the risks on human and environmental health (Duke *et al*., 2001).

Due to the increased risk of using chemical herbicides, a number of researchers have sought alternative methods of weed management. Using allelopathy is one of the alternatives to reduce these problems and to reach sustainability in agriculture and maintain an unpolluted environment by reduce the usage of the herbicides. The main aim of allelopathy is to decrease environmental pollution and maintain the

ecological balance of the organisms through decreasing use of chemical herbicides (Patil, 2007).

Allelopathy is a natural approach to weed control which is based on the beneficial or harmful effects of one plant upon another and can involve either cultivated or wild plant species (Rice, 1984). The allelopathic effect of these plants is produced through chemical compounds such as; glucosinolates, phenolics and alkaloids from the plant which are released through volatilization, leaching and exudation (Weston, 2005). During the last three decades the potential influence of allelopathy in agriculture has been defined and discussed in detail (Qasem and Foy, 2001; Singh *et al*., 2001; Weston and Duke, 2003; Weston, 2005).

Allelopathy may be used for pest; weed, insect, nematode, and pathogen control (Farooq *et al*., 2011a). Allelopathic plants used as mulch, cover crops, in rotational growing and as water extracts can reduce weeds, improve soil quality and increase yield significantly. (Awan *et al*., 2009 and Naseem *et al*., 2009).

Allelopathy has obtained great attention from several investigators worldwide (Duke *et al*., 2001); crop plants such as brassicas (Al-Khatib and Boydston, 1999; Narwal, 2001; Roshdy *et al*., 2008), sesame (Kumar and Varshney, 2008), sunflower (Nikneshan *at el*., 2011) and sorghum (Cheema *at el*., 2008). Researchers have reported that all plant parts including stem, roots, leaves, flowers, rhizomes, seeds and fruits have ability to produced allelopathic substances (Alam, 1993).

In recent years *Brassica spp* have become increasingly important crops due to their high production of oil (McKevith, 2005); they also contain chemical compounds that can be used in weed control (Narwal, 2001). Rapeseed (*Brassica napus*) is cultivated in more than 120 countries in the world and contains 40- 47% oil and is also a rich source of protein 25 % (Roshdy *et al*., 2008). Previous studies have shown that several members of the Brassicaceae family have a number of

biologically active compounds including glucosinolates and their hydrolysis products thiocyanates and isothiocyanates, which have the ability to reduce seed germination and plant growth (Al-Khatib and Boydston, 1999).

1.2. Allelopathy definition and history

In 1937, Hans Molisch from Austria used the term of allelopathy for the first time. He created the term from two Greek words; 'allelon' which means 'of each other' and 'pathos' which means 'mutual harm' or 'to suffer' the injurious effect of another (Lux-Endrich and Hock, 2004). The International Allelopathy society in 1996 defined allelopathy as “Any process involving secondary metabolites produced by plants, micro-organisms, viruses and fungi that influence the growth and development of agricultural and biological systems (excluding animals), including positive and negative effects” (Torres *et al*., 1996).

The phenomena of allelopathy has been reported over two thousand years ago. Theophrastus, in the 300 BC, was the first to report the phenomenon of allelopathy and noted inhibition of weeds by chickpea (*Cicer arietinum*) plants (Willis, 1985). Pliny II (Plinius Secundus, 1 A.D) found the exudates from plants such as chickpea, bitter vetch (*Vicia ervilia*) and barley (*Hordeum vulgare*) have effects on other plants and damaged the land (Rice, 1974). Japanese agronomists during 1600`s noticed that red pine (*Pinus densiflora*) had a harmful effect on crops growing under the pine after rain had washed off their leaves (Lee and Monsi, 1963). In the same period a number of English naturalists observed that some plants cannot develop well when they grow under red pine (Weston, 1996).

DeCandolle in 1832 found that “soil sickness” in agriculture was caused by root exudates (Mandava, 1985). This theory was rejected because it was not supported by experimental data. Until 1900`s scientific experiments were not undertaken on

allelopathy, although the scientists had recognized the allelopathic phenomenon in plants two thousand years ago (Rice, 1984).

1.3. Allelochemicals

Allelopathic substances are termed as allelochemicals (Whittekar, 1970; Levin, 1976)**.** Most importantly secondary metabolites known as allelochemicals are produced via two biochemical pathways, acetate and shikimate acid which are involved in the production of secondary metabolites including flavonoids, alkaloids, phenolic acids, terpenoids, coumarins, brassinosteroids, hydroxamic acids, sulfides, cucurbitacins, saponins, chromenes, polyacetylenes, momilactone, glucosinolates, salicylates, jasmonates, carbohydrates and amino acids (Narwal,1994; Chou,1999; Kruse *et al*., 2000; Koul,2008; Jabran and Farooq, 2012; Farooq *et al*., 2013).

Allelochemicals have been found in many different plants (Table 1.1) and in different plant regions including roots, stems, leaves, flower, rhizomes, pollen, fruits and seeds. These chemical compounds enter the soil and environment by decomposition of plant residues, rain leaching from the leaves and stems, volatilization and root exudation (Figure 1.1). Additionally microorganisms may have a role in the production of these chemical substances (Rice, 1984; Einhellig, 1996; Kruse *et al*., 2000). New analytical techniques used in bioassays, isolation, extraction and identification of the compounds responsible for allelopathic interaction has increased knowledge about allelochemicals area and their allelopathic effects (Willis, 1997).

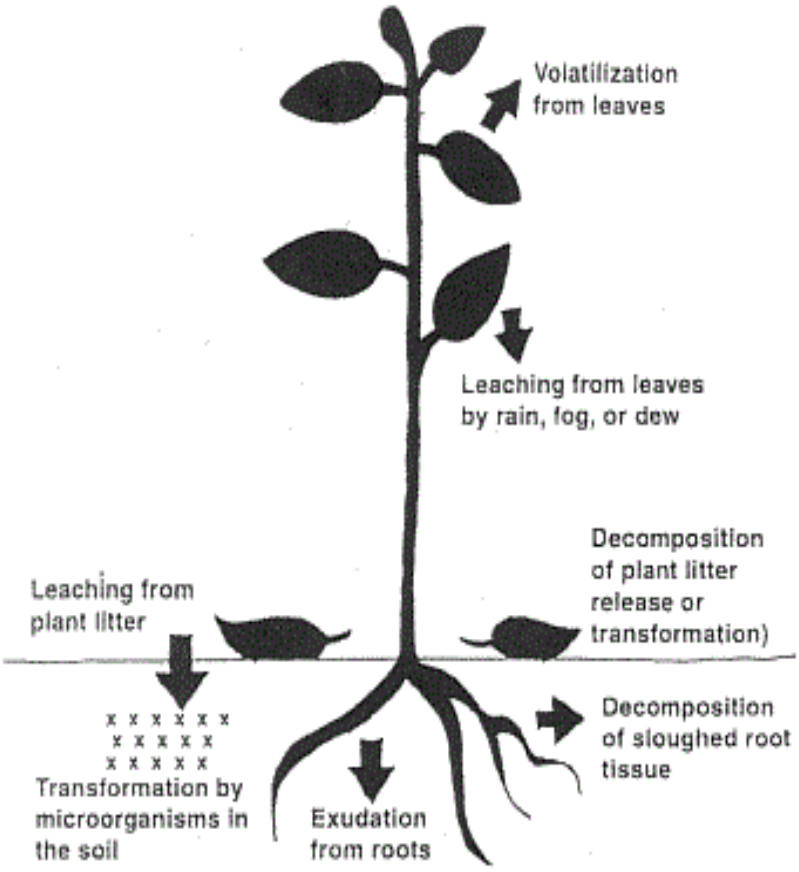

Figure 1.1 Routes by which allelochemicals release into the environment (Rice, 1984)

Table 1.1. Allelochemicals revealed in different plant species

Plant species	allelochemicals	References
Brassica *spp*	Caffeic acid, ferulic acid, vanillic acid, thiocyanates, Chlorogenic acid, isothiocyanates and phenolics acid.	Widmer and Laurent (2006) Velasco *at el*.(2008) Haddadchi & Gerivani (2009
Sorghum *Sorghum bicolor*	Benzoic acid, p- comuaric acid, ferulic acid, chlorogenic acid,	Nimbal *et al* (1996) Alsaadawi and Dayan (2009)
Sunflower *Helianthus annuus*	Chlorogenic acid, isochlorogenic acid, naphthol, scopolin, annuinononones, helivypolide D	Macias *et al*. (1998) Macias *et al*. (1999) Anjum and Bajwa (2005)
Wheat *Triticum aestivum*	p-hydroxybenzoic, vanillic, cis-p-coumaric, syringic, cis-ferulic, trans-p-coumaric, and trans-ferulic acids	Wu *et al*. (2000) Wu *et al*. (2001)
Barley *Hordeum vulgare*	Hordenine	Kruse *et al*. (2000)
Rye *Secale cereale*	2,4-dihydroxy-1,4(2H)-benzoxazin-3-one (DIBOA) and 2 (3H)-benzoxazolinone (BOA).	Jane *et al.* (1987) Burgos *et al*. (2004) Schulz *et al*. (2013)
Maize *Zea mays*	6-methoxy-2-benzoxazolinone (MBOA) and 2, 4-dihydroxy-1, 4(2H)-benzoxazin-3-one (DIBOA).	Maaq *et al*. (2014)
Tobacco *Nicotiana tabacum*	cinnamic and benzoic acids	Huiyong *et al*. (2014)
Rice *Oryza sativa* L	Phenolic acids, fatty acids, indoles and terpenes	Noguchi (2012)
Buck wheat *Fagopyrium esculentum*	Fatty acids and phenolic acid	Weston (1996) Golisz *et al*.(2007)
Oat *Avena sativa* L.	Phenolic acids and Scopoletin	Dimberg *et al*.(2005) Bertoldi *et al*.(2009)

1.4. Release of allelochemicals from plants

More recently allelopathic inhibition of weeds has received greater attention and during the last three decades the potential influence of allelopathy on agriculture has been defined and discussed in detail (Qasem and Foy, 2001; Singh *et al*., 2001; Weston, 1996; Weston and Duke, 2003). Using allelopathic plants by different methods, can reduce weeds, improve soil quality and increase yield significantly (Narwal, 2000; Awan *et al.,* 2009; Naseem *et al*., 2009).

Inhibition of weeds, using allelochemicals has been noted in a wide range of crops such as barley (*Hordeum vulgare*), alfalfa (*Medicago sativa*), brassica (*Brassica spp.*), sorghum (*Sorghum spp.*), sunflower (*Helianthus annuus*), oat (*Avena fatua*), rye (*Secale cereale*), rice (*Oryza sativa*), tobacco (*Nicotiana tabacum*), sesamum (*Sesamum indicum*) wheat (*Triticum aestivum*) and clovers (*Trifolium spp.*) (Narwal, 1996; Weston, 1996; Narwal *et al*., 1998; Weston, 2005).

Many studies have reported reduced germination, growth, development and yield of weeds and crops due to the release of allelochemicals when other crops were growing in the same field (Batish *et al*., 2001; Awan *et al.,* 2009; Naseem *et al*., 2009). For example, Uremis *et al*. (2009) found that shoot and root growth of *Amaranthus retroflexus* L. (redroot pigweed), *Solanum nigrum* L. (black nightshade), *Portulaca oleracea* L. (common purslane), *Physalis angulata* L. (cutleaf ground cherry) and *Echinochloa colonum* (L.) Link. (junglerice) were significantly affected by allelopathic potential of 25 rapeseed cultivars. Putnam and Duke (1978) first explored the possibility of using allelopathic crops to inhibit weed growth in crop production. They suggested weed management using several techniques including the use of rotational, intercrops and cover crops. Yeganehpoor *et al*. (2015) found that weed biomass was significantly affected by cover crop (clover, hairy vetch, basil and dill) and sowing date interaction.

Allelochemicals are found in a range of plant tissues and are not restricted to specific parts of the plant (Sodaeizadeh *et al*., 2009; Gella *et al*., 2013). Moreover, the highest concentrations of these chemicals are most often stored in the leaves and roots, although allelochemicals may be located in flowers as well (Schoonhoven *et al*., 2005; Jafariehyazdi and Javidfa, 2011). Results showed that different levels of germination inhibition rates have been obtained by using fresh shoot and root extracts of *Brassica* species. The greatest suppression of germination were observed when treated by *Brassica napus* shoot extract and *Brassica campestris* root extract and the seed germination inhibited up to 58.7% and 54.3% respectively. Different substances such as allelochemicals and secondary metabolites from any plant species are released into the environment through various means including, extraction and decomposition, leaching, root exudation and volatilization. The toxicity of extracts, leachates and decomposed residuals depend on the type and concentration of chemical compounds available in them (Chang and Chang, 2015).

In recent years *Brassica spp* has been receiving more attention from researchers due to their allelopathic effect on germination and growth of weed species (Nawal, 2000). Members of the Brassicaceae family produce such as caffeic, ferulic, chlorogenic and vanillic acids, thiocyanates and isothiocyanates (Table 1.2). All of these compounds have been recorded to have suppressive effect on weed germination and seedling growth (Bell and Muller, 1973; Putnam, 1988; Al- Khatib *et al*., 1997).

Branca *et al*. (2002), reported that high levels of glucosinolates were found in several *Brassica spp*., which have ability to reduce the germination and seedling growth of plant species (Al-Khatib and Boydston, 1999). An experiment was conducted to determine the effect of using *Brassica juncea* and *Sinapis alba* as a green manure on *Vigana unguiculata* , and the results showed that after 8 weeks

the *Vigana unguiculata* biomass was 118 g/m^2 compared to the 413 g/m^2 in control treatment (Norsworthy *et al*., 2005). In a glasshouse and laboratory experiment conducted by Tawaha and Turk (2003), to determine the effect of black mustard (*Brassica nigra* L.) on wild barley germination and growth. Fresh black mustard residue and water extracts from leaves, stem, flower and root were applied to the wild barley. It was found that fresh black mustard residue reduced the plant height and weight of wild barley significantly. Also, wild barley hypocotyl length, hypocotyl weight, radicle weight, seed germination, and radicle length were reduced by 44, 55, 57, 63 and 75 %, respectively when black mustard water extract was compared with the control.

Table 1.2. Physiological mechanism of action of *Brassica napus* (L.) allelochemicals

Allelochemicals	Inhibition	References
Caffeic acid	Reduction in seed germination and growth	Widmer and Laurent (2006)
Ferulic and vanillic acids	PO_4, NO_3, SO_4, N, P, K, Fe uptake	Al- Saadawi *et al*. (1986)
Chlorogenic acids and isothiocyanates	Reduction in seed germination	Vaughn and Berhow (1999)
Phenolics	Reduction in seed germination	Haddadchi & Gerivani (2009)

Similar results were obtained by Turk and Tawaha (2003), when they carried out an experiment in Jordan to suppress germination and seedling growth of wild oat (*Avena fatua* L.) by using black mustard (*Brassica nigra* L.). They found that water extracts made using different plant parts from *B. nigra* (leaf, stem, flower and root) at different concentrations significantly affected germination, dry weight, shoot and root length. They also found that effectiveness increased significantly with increasing the water extract concentration of different parts of *B. nigra* (L.).

Similar results were achieved by Uremis *et al*., (2009) in field and laboratory conditions when they used the residues of six brassica species to test their effect on Johnsongrass (*Sorghum halepense* L.) in Turkey. Different parts of the field were used each year to cultivate Brassicaseae species (round white radish, garden radish, black radish, Little Radish, turnip and rapeseed). They found that Johnsongrass rhizomes were suppressed by rapeseed and Little Radish in the field. However, Johnsongrass was most effectively suppressed by Little Radish extract and garden radish extract under controlled conditions.

In the USA, Boydston *at el*. (2008) added mustard (*Sinapis alba*) seed meal (MSM) to the soil surface of pots (113, 225, 450 $g.m^{-2}$) to investigate the effect of MSM on several weeds (*Poa annua* L., *Stellaria media* L., *Phlox paniculata* L. and *Coreopsis auriculata* L.). The number of *Stellaria media* L. plant decreased by 61%, 74%, and 73% respectively after 8 weeks and the number of *Poa annua* L. decreased by 60%, 86%, and 98%, respectively. However, the researchers did not mention the temperature at which the experiment was carried out in the field environment or in the glasshouse and in which season. These factors would have an effect on the results.

Although a number of publications have shown that brassica species have an effect on the germination and growth of weeds, field studies conducted in US revealed that brassica cover crops had little effect on redroot pigweed (Haramoto and Gallandt, 2005). This could be attributed to differences in environmental conditions. For example, Tang *et al*. (1995) noted that concentration of phenolics and terpenes were increased by water- deficit stress. Also, they found that this increase may occur during nutrient or temperature stress.

Brassica spp. cover crops and weed control is well reviewed by Boydston and Al-khatib (2006) and Haramoto and Gallandt (2004). They focus on plant allelopathic as the tool of weed control, an also on the hydrolysis produced by glucosinolates as the allelochemicals responsible. One of the secondary plan metabolites is glucosinolates which is found in *Brassica spp*., and myrosinase enzyme can hydrolysis the glucosinolates into toxic products like isothiocyanates, that have ability to control weed seeds (Brown and Morra, 1997; Al-Turki and Dick, 2003). Seed germination and seedling growth of numerous of weeds were inhibited by isothiocyanate (Brown and Morra, 1997; Al-Turki and Dick, 2003; Norsworthy *et al*., 2006; Bangarwa *et al*., 2010).

1.4.1. Volatilization

In volatilization, the harmful chemical compounds are passed into the environment in the form of a gas from the leaves and then are absorbed by the plants nearby. Such volatiles may affect normal cellular processes (Chang and Chang, 2015). It was reported that many plant species have an ability to release chemical compounds into the air in the form of gas through leaf stomata. The mechanism of release of allelochemicals might be more significant under stress conditions (Rice, 1974). For instance, the allelochemicals that were released from the plant tissues may move through the atmosphere and be absorbed by other plants, or chemical compounds may pass to the soil and solubilize in the soil solution, this mechanism is known as a biofumigation process (Haramoto and Gallandt, 2004). Biofumigation is the release of volatile allelochemicals, and their subsequent hybridization, from decaying *Brassica spp* tissue into the soil to inhibit pests and weeds (Kirkegaard and Sarwar1998; Matthiessen and Kirkegaard, 2006; Haidar, 2013). For example, using *Sinapis alba*, *Brassica napus*, *Brassica juncea* as cover crops significantly reduced winter- growing weeds due to their biofumigation potential (Fourie *et al*.,

2014). Additionally, *Brassica spp* residue has been shown significant biofumigation impact on disease when incorporated into soil, as a result of conversion of glucosinolates to isothiocyanates (Hartz *et al*. 2005). Isothiocyanates are compounds produced under enzymatic hydrolysis of glucosinolate that are characterized by volatile (Higdon *et al*., 2007; Bangarwa *et al*., 2011). An experiment was conducted by Bangarwa *et al*. (2010) to investigate the effect of different concentrations of phenyl isothiocyanate (ITC) and exposure period on purple nutsedge tuber viability. Purple nutsedge tuber viability was reduced by up to 97% by using phenyl ITC concentration of 676 ppm in soil for 3 days in a closed environment compared with the control. Similar work was carried out by Norsworthy *et al*. (2006) in a greenhouse to determine the effects of applying five concentrations of isothiocyanates (ITCs) 0, 100, 1000, 5000, and 10 000 nmol g^{-1} to soil in closed jars for 72 h to prevent gaseous losses on purple and yellow nutsedge. Purple and yellow nutsedge shoot density and shoot biomass were inhibited by all ITCs concentrations. Petersen *et al*. (2001) reported that aqueous aryl-ITCs solutions were most effective on germination of weed seeds and the sensitivity of different weed species to ITCs mostly depended on the size of seeds, and that small seeds may be more sensitive.

Zhang *et al*. (2012) noted some volatile allelochemicals were released from fresh leaf tissues of the Crofton weed (*Ageratina adenophora*) and these compounds had a significant effect on seedling growth of rice. Wang *et al*. (2005) identified that thirty-eight allelochemicals were released from Giant ragweed (*Ambrosia trifida*) some of which passed into the air such as bicyclic monoterpenes, and affected other plant species. They also found that maize and wheat germination and growth was significantly reduced by volatile allelochemicals of Giant ragweed (*Ambrosia trifida*). Furthermore, Kim & Kil (2001) showed that volatile allelochemicals released from

leaves of tomato (*Lycopersicon esculentum*) had a significant effect on some crop species growing in the nearby.

1.4.2. Root Exudation

Several published papers show that roots of some plant species can exude allelochemicals. This process is termed root exudation, many of the exudates chemicals from plants are toxic therefore they may be used as potential herbicides (Drake *et al*., 2013).

Root exudates contribute significantly in allelopathy because they contain a lot of chemical compounds. Seed germination, root and shoot length and nutrient uptake were shown to be reduced due to many allelochemicals released from roots (Yu and Matusi, 1994; Weston, 1996). Many factors including nutrition, moisture, plant age and light may affect the quality and quantity of allelochemicals (Einhellig, 1987).

Einhellig and Souza (1992) demonstrated that *Abutilon theophrasti*, *Datura stramonium*, *Amranthus retoflexus*, *Setaria viridis*, *Digitaria sangunalis* and *Echinochloa crusgalli* growth were reduced by root exudates of sorghum due oxidization of dihydroquinone to ap-benzoquinone (sorgoleone). Additionally, black mustard root exudates suppressed seed germination and seedling growth of *Phalaris paradoxa* and *Sisymbrium irio* due to autotoxic effects of isothiocyanates (AL-Sherif *et al*., 2013).

1.4.3. Leaching from plant tissue

Leaching phenomenon was noted for the first time in 18th century (Rice, 1984). This term is known as a ‘removing allelochemicals from plants ’ which take place in living plants or in residues of the same plants into the environment because of rain, fog, dew and snow (Kumari and Kohli, 1987). Many allelochemicals that are released by

the leaching process include organic substances such as phenolic acids, terpenoids and alkaloids and these substances may have toxic effects on plants and the microorganisms in the neighborhood (Race, 1984).

Creamer *et al*. (1996) found that leaching from Crimson clover (*Trifolium incarnatum* L.) tissues reduced the emergence of Eastern black nightshade (*Solanum ptycanthum* Dun), while the germination of yellow foxtail (*Setaria glauca* L.) was suppressed by rye (*Secale cereale* L.) and barley (*Hordeum vulgare* L.). In India researchers found that mesquite (*Prosopis juliflora*) had an ability to inhibit the growth of other plants around it due to the leaf leachate which containing phenolic acids (Geol *et al*., 1989).

In Japan the effects of leaching from dry leaves and exudates from the roots of 71 ground cover plant species on lettuce (*Lactuca sativa*) were tested. Results showed that seven species, Moss pink (*Phlox subulata*), trefoil (*Oxalis brasiliensis*), red spiderlily (*Lycoris radiata*), creeping thyme (*Thymus serpyllum*), European pennyroyal (*Mentha pulegium*), Roman chamomile (*Chamaemelum nobile*) and Star-of-Bethlehem (*Ornithogalum umbellatum*) had strong effects on shoot and root elongation of Lettuce seedling. Based on these results these cover crops were selected for the management of three weed species; live amaranth (*Amaranthus lividus*), southern crabgrass (*Digitaria ciliaris*) and common lambsquarters (*Chenopodium album*). Root length of all the tested weed species were reduced by leachate from trefoil and red spiderlily and root exudates from moss pink, trefoil and creeping thyme (Shiraishi *et al*. 2002).

A series of field trials were conducted by Cheema *et al*. (2008) to examine the allelopathic effects of sorghum on weed control and wheat production. They observed that by applying 10% w/v water leachate from aerial parts of *Sorghum bicolor* after 30 and 60 days of sowing can decrease weed biomass by as much as 49% and wheat yield increased over 20% compared with the control. They found

that water leachate from sorghum has a high potential to increase weed control and yield of wheat. This method for weed management has great economic and environmental benefits to crop cultivation.

1.4.4. Decomposition of plant residues

During the process of decomposition of plant residues such as dried stems, leaves, roots, fruit and seeds, allelochemicals may be released into the environment and these allelochemicals may influence the growth and development of other plants (Chou 1999; Kruse *et al*., 2000; Tesio and Ferrero, 2010). In recent times, researchers have observed the ability of some cover crops and their residues to inhibit weeds (Liebman and Davis, 2000; Petersen *et al*., 2001; Brennan and Smith, 2005).

However, Haramoto and Gallandt (2005), found that some allelopathic cover crops including rapeseed and yellow mustard inhibit both weeds and subsequent crops. A similar result was obtained by Ackroyd *et al*. (2011), who observed that oilseed radish (*Raphanus sativus* var. oleiferus), Indian mustard (*Brassica juncea*), and white mustard (*Sinapis alba*) were used as green manures, germination percentage and radicle elongation of muskmelon (*Cucumis melo*) were reduced.

Moreover, smother cropping includes the use of a living plant to decrease the growth and development of weeds mainly through competition for resources effect or this effect can be physical as well as allelopathic (Teasdale, 1998). In a study conducted by Putnam *et al*. (1990), smother crops such as black mustard, buckwheat, rye, sorghum and wheat were used for weed control.

Certain crops suppress growth of several weed species by releasing phytotoxins from crop residues reducing weed seed germination (Bhadoria, 2011). However, the main concern about the crop remainders is their toxic effect on other crops (Thorne

et al., 1990). A field experiment was conducted by Khaliq *et al*. (2010) to test the weed control potential by using a mixture of allelopathic crop residues and their effects on maize yield. Residues of sorghum (*Sorghum bicolor L.*), sunflower (*Helianthus annuus*), rice (*Oryza sativa*) and brassica (*Brassica campestris* L.) in various combinations were mixed in the soil at 5 and 7.5 t ha^{-1}. The results showed that the mixture of sorghum, sunflower and brassica residues at 7.5 t ha^{-1} reduced the density and dry weight of horse purslane (*Trianthema portulacastrum* L.) and purple nutsedge (*Cyperus rotundus* L.) up to 90% compared with the control . These results indicate that mixing crop residues with soil may help as an important tool for weed management in maize fields.

Khaliq *et al*. (2011a), investigated the allelopathic potential of sorghum cv. JS-263, sunflower cv. Hysun-33 and brassica cv. Rainbow residues applied individually or in combination to the soil for horse purslane (*Trianthema portulacastrum*) management. They observed that the crop residue incorporated into the soil significantly reduced the emergence index, final germination percentage, shoot and root length, leaf and root score and leaf area per plant. Horse purslane seedlings were reduced by 71% following exposure to the mixture of sorghum and sunflower residues. Thus, allelopathy can play an effective role in suppressing weeds through soil mixing with crop residues.

For example, an experiment was conducted to investigate the influence of sorghum, sunflower and brassica residues on purple nutsedge (*Cyperus rotundus*). It was found that the combinations of residues were more effective in inhibiting purple nutsedge than the individual crop residues. The mixture of Sorghum and brassica residues did not allow any tuber to sprout. In addition, shoot and root length, shoot and root dry weight were reduced significantly (Matloob *et al*., 2010).

In addition, Khaliq *et al* (2011b), investigated the allelopathic potential of three crops residues; sorghum, sunflower and brassica in rice (*Oryza sativa* L.) and jungle rice (*Echinochloa colona* [L.] Link) as associated grass weed. Crop residues of the three species were chopped into small pieces and mixed with soil by 6 g kg^{-1} soil (12 t ha^{-1}) and a control treatment without residues was used. The germination of jungle rice was delayed by mixing crop residues with soil. Residue incorporation delayed germination time by 50% until emergence, mean emergence time, emergence index, and final germination percentage. Also, final germination of rice and jungle rice were inhibited by 11 to 15% and 11 to 27% when crop residues were added to the soil individually and by 18 to 22% and 8 to 34% with a mixture of crop residues, respectively. Crop residues affect shoot length significantly (25 to 100% and 14 to 44%) and root lengths (22 to 100% and 10 to 43%) of rice and jungle rice, respectively. Also, the shoot and root dry weight of rice and jungle rice were reduced significantly.

In organic agriculture farmers frequently use crop species in rotation or as cover crops to control weeds (Bond and Grundy, 2001). Weston (1996) suggested that crop rotation has a greater influence on weed species control than tillage and additionally reduces weed densities. Japanese farmers found that using beans in spring, buckwheat in summer then wheat in winter helped to increase the yield of wheat and decrease weeds. They reported that beans can increase the nutrient levels in the soil, whilst buckwheat has the ability to inhibit weeds (Khan *et al*., 2005).

1.5. Factors that affect allelopathy

Several factors affect allelochemical production in plants including; temperature, light, water stress, mineral deficiency and age of plant, (Rice, 1984). Challker-Scott (1999) and Kopsell and Kopsell (2006) found that both nutrient and environmental

factors may affect growth, primary metabolism and a number of secondary pathways such as the synthesis of anthocyanins and carotenoids.

1.5.1. Light effects

Light is one of the factors that may affect the quantity of allelochemicals released by plants (Mkula, 2006). Cooner (1987), suggests that the increased concentration of allelochemicals in the presence of light could be directly related to an increase in rate of photosynthesis.

Several studies have shown that light quality and quantity may affect glucosinolate concentrations in *Brassica* species. Yang *et al*. (2009) reports that under normal light intensity the concentrations of gluconapin, glucobrassicanapin, glucobrassicin, neoglucobrassicin, 4-methoxyglucobrasicin and gluconasturtiin were significantly increased. Moreover, Engelen-Eigles *et al*. (2006), observed that in watercress (*Nasturtium officinale* R. Be) the concentration of gluconasturtiin was increased by approximately 25- 40% when grown under metal halide light enriched with red compared with far-red light. In a study conducted by Pérez-Balibrea *et al*. (2008), they reported that in *Brassica oleracea* plants grown in dark conditions (darkness produced by wrapping the sprouting trays with domestic aluminium foil) contained lower total glucosinolates compared to those grown in light condition (16 h light / 8 h dark). Furthermore, Mølmann *et al*. (2015), reported there is a link between contents of glucosinolates and long photoperiod, whereas, concentrations of gluciberin and glucoraphanin significantly increased in *Brassica oleracea* tissues under long day photoperiod. However, Steindal *et al*. (2015) found that the content of glucoiberin reduces by up to 45% during a long day with high temperature in *B. oleracea* tissues.

1.5.2. Temperature effects

Temperature may also affect accumulation of allelopathic compounds in plants. Plants facing resource limitation such as nutrient deficiencies and different levels of temperature produce larger amount of allelochemicals than those growing in good environmental conditions (Tang *et al*, 1995). Justen and Fritz (2013) reported that the glucosinolate levels of *Brassica rapa* increased by increasing the temperature**.** In a study conducted by Engelen-Eigles *et al*. (2006) gluconasturtiin concentration of watercress (*Nasturtium officinale*) was found to increase by at least 50% when the watercress plants were grown during days of lengthy daylight (16 h) and temperatures were 10 or 15 °C. In addition, in *B. oleracea* tissues the high temperature was significantly reduced the glucoiberin up to 45% (Steindal *et al*., 2015).

1.5.3. Water stress effects

Water stress may affect plant physiology, morphology, and chemistry, which can in turn effect root and shoot growth and production of allelochemicals (Taiz and Zeiger 2010). In addition, water is one of the important factors affecting allelopathy, because it works as a solvent and transporter of allelochemicals in the soil (Reinhardt *et al*., 1999). Several studies have shown that allelochemical concentrations have been affected by moisture availability (Ren, *et al*., 2009; Hosseini and Hassibi, 2011, Kheradmand *et al*., 2014). The concentrations of alkaloids in opium poppy (*Papaver somniferum*) (Szabó *et al*., 2003) and phenolics in purple coneflower (*Echinacea purpurea*) (Gray *et al*., 2003), purple nutsedge (*Cyperus rotundus*) (Tang *et al*., 1995), St. John's wort (*Hypericum perforatum*) (Gray *et al*., 2003) and tomato (*Solanum lycopersicum*) (English-Loeb *et al*., 1997) increased under drought stress conditions.

The concentration of phenolic compounds in leaf exudate of false yellowhead (*Dittrichia viscosa*) was increased under drought stress, however, the total phenolics were reduced because of reduced leaf area (Karageorgou *et al*., 2002).

In field experiments conducted in Germany Zhang *et al*. (2008) determined the effect of growing season (spring-summer, summer-autumn and autumn-winter) and water supply by three levels (25%, 50% and 75% of available soil water) on glucosinolate concentrations in turnip (*Brassica rapa*) roots was determined. It was found that the total glucosinolate concentration in turnip root was 1774-3221 µmol kg^{-1}, while fresh matter and gluconasturtiin showed the highest concentration 1004-1628 µmol kg^{-1} fresh matter in turnip roots. In spring- summer concentrations of total glucosinolates increased up to 52% and 47%, aliphatic glucosinolates up to 60% and 131%, and aromatic glucosinolates up to 47% and 21% under 25% available soil water treatment as compared with 50% and 75% available soil water treatments, respectively.

On the other hand, glucosinolate concentration in Brassica species increased under water stress such as; *Brassica oleracea* L. var. capitata, *Brassica napus* L., *Brassica rapa* ssp. rapifera L. and *Brassica carinata* (Radovich *at el*., 2005; Champolivier and Merrien, 1996, Jensen *et al*., 1996; Zhang *et al*., 2008; Schreiner *et al*., 2009). Water stress may reduce the vegetative growth of brassica plants with the subsequent increase of plant secondary metabolites by reducing primary metabolism (Jones and Hartley, 1999).

Glucosinolates increase under water deficiency conditions has been documented as part of the plant reaction to water stress through the process of osmotic adaptation (Schreiner *et al*., 2009). However, Gutbrodt *et al*. (2012) reported different results; that concentration of total glucosinolates in *Brassica oleracea* was not affected by high water stress (30% of the amount of water received by well-watered plants), and also in *Brassica napus* under mild water stress (Jensen *et al*.,

1996), while the glucosinolate concentration in *Brassica oleracea* significantly reduced under water stress conditions (Robbins *et al*., 2005; Khan *et al*., 2011). Thus, the intensity and length of water deficiency seems to be a significant factor in the increase of glucosinolates, as well as the plant growth stage when the water stress is applied (Hale *et al*., 2005).

Moreover, Khan *et al*. (2010) noted that *Brassica oleracea* grown for two weeks under water stress were significantly smaller and showed reduced concentration of total glucosinolates compared to glucosinolate contents of well-watered plants, while glucosinolate content increased slightly in plants grown under normal watering conditions. In addition, a significant reduction in indolyl glucosinolate was identified in water-deficient plants; however aliphatic glucosinolate reduced slightly. Mailer and Cornish (1987) studied the effect of water stress on glucosinolate levels in the seed meal of *Brassica napus* and *Brassica* rapa. The results showed that in seeds of both cultivars the glucosinolate concentrations were increased significantly from 18.2 $\mu mol.g^{-1}$ in unstressed plants to 35.0 $\mu mol.g^{-1}$ under the influence of water stress.

1.5.4. Plant age

Several studies determined that plant species may produce different quantities of chemical compounds at different growth stages (Rice, 1984). Reinhardta and Bezuidenhouta (2001) investigated the effect of using water extract from plant shoot and tubers of *Cyperus esculentus* collected at immature and mature stages on lettuce. They found that germination of lettuce was reduced by using a 2% extract dilution from both plant parts and stages of development. However, a 5% dilution water extract from immature plant shoot completely inhibited lettuce germination as compared with the control.

Jafariehyazdi and Javidfar (2011) studied the effect of allelopathic potential of *Brassica napus*, *Brassica rapa* and *Brassica juncea* on sunflower seed germination and seedling growth. Water extracts collected of three species at two stages (full flowering and straw) were diluted to 10, 20, 30 and 40%. It was found that there was a highly significant difference between the two stages and also between different dilutions. Germination, root and shoot length and fresh and dry matter weight were significantly affected by water extracts compared with the control.

1.5.5. Variation in allelochemical in plant tissues

Race (1984) has reported that the greatest concentrations of allelochemicals were found mostly in leaves and sometimes in the roots or seeds. In a study conducted by Muhammad and Majeed (2014), the allelopathic potential of fresh water extracts and air dried water extracts of root, shoot and leaves of sunflower on germination and seedling growth of wheat (*Triticum aestivum* L.) and maize (*Zea mays* L.) was investigated. Results showed that seed germination, growth and dry biomass of seedlings of wheat and maize were significantly reduced by water extracts from all plant parts. Water extracts from the leaf decreased seed germination of wheat significantly by 15.21%, plumule and radical growth were reduced by 21.66 and 28.44% when compared with extracts from other parts including root and shoot.

A laboratory experiment was conducted in Malaysia by Toosi and Baki (2012) to examine the allelopathic potential of *Brassica juncea* extract in different concentrations 10.8, 14.28, 18 and 30 gL^{-1} from different parts (leaf, stem and root) on radish (*Raphanus sativus* L.) and barnyard grass (*Echinochloa crus-galli* [L.] Beauv.). Leaf ethanol extract at all concentrations suppressed the germination of radish seeds. Additionally, root and shoot growths of barnyard grass and radish seedlings were strongly affected by the leaf stem and root extracts. Although the

authors obtained good results from this experiment, the light in the growth chamber may have affected germination and seedling growth as well.

Dry weight of weeds; *Phalaris minor*, *Chenopodium album*, *Coronopis didymus*, *Rumex dentatus* and *Medicago polymorpha* were significantly reduced by application of sunflower root, stem and leaf extracts (Anjum *et al*., 2005). An experiment was conducted in the lab to investigate the allelopathic potential of water extracts of some weed species: *Amaranthus hybridus*, *Parthenium hysterophorus*, *Datura stramonium* and *Argemone mexicana* including samples from different parts (leaf, stem and root), and the effect on seed germination, seedlings growth and biomass production of wheat cultivars; HAR–1685 and Durum wheat. Leaf extract of *P. hysterophorus* highly reduced the seed germination of wheat by up to 22%. Also, they observed that radicle length of wheat seedlings was inhibited significantly by the same water extract compared to the water extract from other parts (Gella *et al*., 2013).

Several studies showed that allelochemicals, at high concentrations may be more effective; however, at lower concentrations these allelochemicals might stimulate the growth of other species (Narwal, 1994). Turk and Tawaha (2002) evaluated the effect of different concentrations of water extracts from different parts of black mustard (leaves, flowers, roots and mixture) on lentil germination and seedling growth. The results showed that lentil germination decreased by increasing the concentration.

Also, Turk *et al*. (2005) evaluated allelopathic effects of black mustard from different plant parts (leaf, stem, flower and root) on radish (*Raphanus sativus*) germination and seedling growth. They found that radish seed germination and seedling growth were inhibited significantly by all water extracts compared with the control. Increasing the water extract concentrations of individual plant parts significantly inhibited seed germination.

A laboratory experiment was conducted to investigate the allelopathic potential of different concentrations (0, 50 and 100%) of rice straw extract on germination and seedling growth of different plants. The results showed that 100% rice straw extract significantly reduced the germination and growth of plants compared with 0 and 50% rice straw extracts. *Ipomoea batatas*, *Rumex dentatus* and *Convolvulus arvensis* were most affected by the rice extracts (Afridi *et al*., 2014). In additional work Jamil *et al*. (2009) determined the effect of applying different concentrations of sorghum aquatic extract alone and mixed with aquatic extracts of other plants: sunflower, eucalyptus, sesame, brassica and tobacco, on wild oat and canary grass (*Phalaris minor*). The results show that applying a high concentration (12 Lha^{-1}) from each of sorghum and sunflower extracts was more effective than other mixtures, and wild oat dry matter decreased by up to 42–62%, and canary grass by up to 36–55% compared with 6 (Lha^{-1}).

An experiment was conducted to investigate bio-herbicidal potential of sorghum and sunflower extracts on germination and seedling growth of dragon spurge (*Euphorbia dracunculoides* L.). Different concentrations (0, 25, 50, 75, and 100%) of aqueous extracts, were used individually and in mixture. The results showed that applying sorghum and sunflower water extracts individually and in mixture and their different concentrations delayed germination of *Euphorbia dracunculoides*. Mixture of Sorghum and sunflower water extracts at 100% concentration decreased seed germination by 92%, while applying water extract from sorghum was more effective on *Euphorbia dracunculoides* germination compared with sunflower water extract and the germination was reduced by up to 88% and 80% respectively.

Greenhouse and laboratory experiments were conducted by Ashrafi *et al*. (2009) to examine the effect of barley water extract from different parts (leaf, stem, flower and root) in different concentrations (4, 8, 12, 16, and 20 g of tissue per 100 ml of water) on germination and seedling growth of quack grass (*Agropyrum repens*). Shoot and

root length, shoot and root weight and seed germination quack grass were reduced by barley extracts compared to the control. The inhibition of quack grass germination, seedling length and weight increased significantly by increasing the water extract concentrations from 4 to 20 g per 100 ml of water of all barley parts. Water extracts from leaves and flowers were more effective when compared with water extracts from other parts.

1.6. Mode of action of allelochemicals

According to Rice (1974), allelochemicals may affect plant growth by affecting a number of physiological processes such as, cell division and elongation, mineral uptake, stomatal opening and photosynthesis, membrane permeability, protein, phytohormone induced growth, change in lipids, seed germinations, change in organic acid metabolism, inhibition of enzymes and effect on xylem. In addition, Rice (1984) describes the modes of action of allelochemicals widely, modes of action have also been reviewed in detail by (Alonzo, 1985; Inderjit *et al*., 1996; Al-Khatib *et al*., 1997; Krishana *et al*., 1998; Inderjit and Mallik, 2002; Turk and Tawaha, 2003; Macias *et al*., 2004; Bainard *et al*., 2009; Chon and Nelson, 2010 and Hui Li *et al*., 2010).

Earlier studies have shown that the allelochemicals release from plants may have different effects on the synthesis, functions, contents and activities of different enzymes. For example, chlorogenic acid, caffeic acid and catechol may have to inhibit the key enzyme λ-phosphorylase that involved in seed germination (Einhellig, 1995). Volatile monoterpenoids (camphor, 1, 8-cineole, betapinene, alpha-pinene, and camphene) from *Salvia leucophylla* showed allelopathic effects on cell proliferation and DNA synthesis root apical meristem of *Brassica campestris* seedlings (Nishida *et al*., 2005). The exudate from *Sorghum bicolor* L. (sorgoleone) reduced the number of cells in prophase, metaphase, and anaphase stages (Hallak

et al., 1999). Soltys *et al* (2012) reported that cyanamide produced by hairy vetch (*Vicia villosa* Roth) was shown a strong allelopathic effect on roots growth of tomato (*Solanum lycopersicum* L.) by modifications in cell division and change in plant hormone levels (ethylene and auxin).

Several studies have found that roots are more affected by allelochemicals than seedlings growth. Cai and Mu (2012) observed that the primary root elongation and lateral root development of *Glycine max* (L.) were inhibited by leaf extracts from *Datura stramonium* L. at high concentration, root hair length and density also decreased. Moreover, Gatti *et al.*, (2010) the growth and development of seedlings of *Sesamum indicum* L reduced and their morphological were change by aqueous extracts of *Artistolochia esperanzae* and the size of root xylem cells was decreased up to 50%, primary root and in the number of secondary roots were changed.

On the other hand, It has been reported that the photosynthesis and respiration significantly affected by allelochemicals which released to the environment by plants (Gniazdowska and Bogatek, 2005). For example, Elisante *et al*., (2013) found that the aqueous seed and leaf extracts of *Datura stramonium* significantly reduced the total chlorophyll content of *Cenchrus ciliaris* and *Neonotonia wightii.* Additionally, the phenolic allelochemicals have ability to impact the respiration of plants through weakening oxygen absorption capacity, meanwhile the effect on photosynthesis may occur by decrease the photosynthetic rate and chlorophyll content (Li *et al*., 2010). Patterson (1981) observed that Caffeic, t-cinnamic, p-coumaric, ferulic, gallic, and vanillic acids at concentrations of 10^{-3} M, significantly reduced the net photosynthetic rate and stomatal conductance and caused high reductions in leaf chlorophyll content of soybean (*Glycine max*). The amount of chlorophyll a, chlorophyll b, total chlorophyll, carotenoids and protein were significantly decreased by aqueous leachate of *Achillea biebersteinii* (Abu-Romman, 2011).

Moreover, total of chlorophyll in Barley (*Hordeum vulgare* L.) leaf was significantly inhibited by aqueous extract of *Malva parviflora* at concentration 100% and *Chenopodium murale* at 75% and 100%. Also, the two weeds was significantly reduced the photosynthesis and *Chenopodium murale* was more effective as compared with *Malva parviflora* (AL-Johani, *et al*., 2012).

1.7. Glucosinolates

Glucosinolates or mustard oils (GLS) are secondary metabolites most noted in species of the *Brassicaceae*, *Capparidaceae* and *Caricaceae* families such as cabbage, radish, broccoli, cauliflower, rapeseed, mustard, horseradish and turnip (Fahey *et al*., 2001; Mikkelsen *et al*., 2002; Rameeh, 2015). Glucosinolates are major secondary metabolites found in all *Brassica spp*. mambers. These chemical compounds play an essential role in plant defense against plant pests. These secondary compounds have a structure which contains a beta-thioglucoside-N-hydroxysulfate linked to a sulfonated aldoxime moiety and changeable side chain derived from amino acids (Figure 3.1). More than 130 natural glucosinolates have been found in several plant families (Fahey *et al*., 2001; Agerbirk and Olsen, 2012; Rameeh, 2015). Based on the side chains, the glucosinolates can be classified into three major groups: (i) aliphatic alkenyl (ii) indolyl (iii) aromatic (Holst and Williamson, 2004; Gimsing *et al*. 2005).

The glucosinolate side chains come from amino acids as the first stages in the biosynthetic pathway. Aliphatic, indolyl and aromatic glucosinolates are derived from methionine, tryptophan and phenylalanine respectively (Mithen 1992; Bennet *et al*. 1993; Schonhof *et al*., 2004; Redovnikovic *et al*., 2008). Each *Brassica* species contains some of the compounds which ultimately determines the profile of glucosinolate produced. Several glucosinolates have been found in rapeseeds

(*Brassica napus* L.) (Fahey *et al*., 2001). Common glucosinolates found in *Brassica spp* are shown in (Table 1.3).

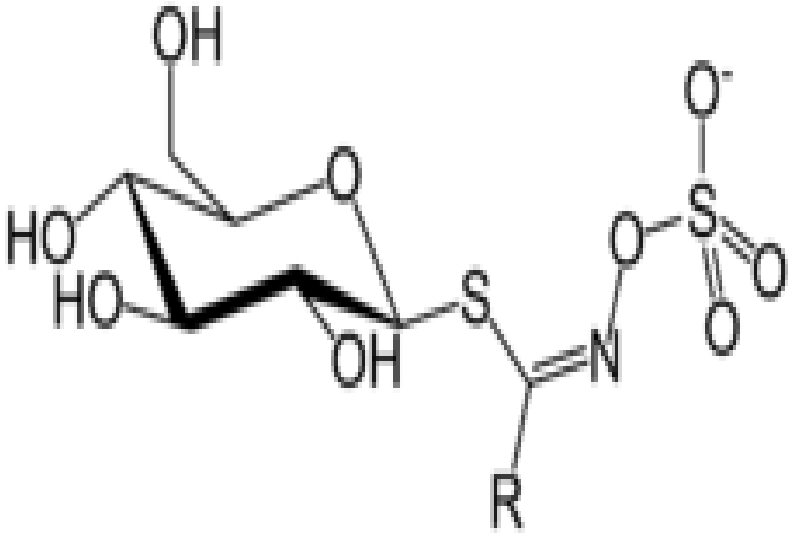

Figure 1. 2. The general structure of glucosinolate (Polat, 2010)

Each *Brassica spp* has different glucosinolate profiles and glucosinolate concentrations. Furthermore, the profile and concentration of glucosinolates found in the tissues of *Brassica spp* and glucosinolate accumulation is affected by environmental conditions such as temperature, water stress, light, soil fertility. Glucosinolate concentration is also affected by the age of plant as well as insect damage, wounding and fungal infection (Booth and Walker 1992; Ludwig-Müller *et al*., 1999; Bartlet *et al*., 1999; Hasegawa *et al*., 2000; Bellostas *et al*. 2007; Alnsour *et al*., 2013; Park *et al*., 2013).

Several researchers noted that the glucosinolates levels and their distribution in the plant were significantly increased in the young leaves, shoot and silique walls and this may refer to the biosynthetic activities which are higher in growth stages (Bennett *et al*., 1995; Bellostas *et al*., 2004). Booth *et al* (1991) found that

glucosinolate levels decreased in the mature leaves, especially at the stage when flowers and during seeds were produced. Moreover, Brown *et al*. (2003) noted that there were significant differences in both concentration and composition of glucosinolates in *Arabidopsis thaliana* tissues. Additionally, concentrations of aliphatic glucosinolates was significantly higher in seeds as compared with other organs, the younger leaves had higher glucosinolate concentrations than older leaves. Malik *et al*. (2010) reported that in wild radish (*Raphanus raphanistrum*) the highest level of glucosinolates was in flowers at the flowering stage.

In addition, based on these findings these authors recommended wild radish should be incorporated into the soil at 50% flowering stage to provide the greatest glucosinolates for weed inhibition. In India, Bhushan *et al*. (2013) observed changes in glucosinolate profiles in ten different genotype of rapeseed mustard. They found that the total glucosinolates were increased significantly at the beginning of the flowering stage to full bloom stage but total glucosinolates reduced during pod maturity stage.

Glucosinolates content in different plant parts have been studied widely. For example, an experiment was conducted by Bellostas *et al*. (2007) to observe the content and distribution of glucosinolates in seeds and seedling of five varieties of *Brassica oleracea*; White cabbage, red cabbage, savoy cabbage, cauliflower and broccoli. The individual glucosinolates concentration and their type showed a differences between the *Brassica oleracea* varieties and plant parts. Concentration of sinigrin decreased while glucobrassicin increased during seedling stage. Glucosinolate concentration was higher in the root, especially in 4 and 7 day old plants compared to the other parts. Bellostas *et al*. (2004) showed that *Brassica rapa* roots contain the highest glucosinolate concentration compared with different plant parts.

Table 1.3. Glucosinolates commonly found in *Brassica* species.

Common name	**Chemical name**	**Molecular Formula**	**R-group**
Sinigrin	2-propentyl (allyl)	$C_{10}H_{16}KNO_9S_2$	Aliphatic
Glucoberin	3-methylsulfinylpropyl	$C_{11}H_{21}NO_{10}S_3$	Aliphatic
Progoitrin	2-hydroxy-3-butenyl	$C_{11}H_{19}NO_{10}S_2$	Aliphatic
Epi Progoitrin	2(S)-Hydroxy-3-butenyl	$C_{11}H_{19}NO_{10}S_2$	Aliphatic
Gluconapin	3-butenyl	$C_{11}H_{19}NO_9S_2$	Aliphatic
Glucobrassicanapin	4-pentenyl	$C_{12}H_{20}NO_9S_2$	Aliphatic
Glucoraphanin	4-methylsulfinyl-butyl	$C_{12}H_{22}NO_{10}S_3$	Aliphatic
Gluconapoleiferin	2- hydroxyl-4-pentenyl	$C_{12}H_{20}NO_{10}S_2$	Aliphatic
4-OH glucobrassicin	4-hydroxyindol-3-ylmethyl	$C_{16}H_{20}N_2O_{10}S_2$	Indolyl
Glucobrassicin	Indol-3-ylmethyl	$C_{16}H_{19}N_2O_9S_2$	Indolyl
Neoglucobrassicin	1-Methoxy-3-indolylmethyl	$C_{17}H_{22}N_2O_{10}S_2$	Indolyl
Sinalbin	4- hydroxybenzyl	$C_{14}H_{19}NO_{10}S_2$	Aromatic
Gluconasturtiin	2-phenylethyl	$C_{15}H_{21}NO_9S_2$	Aromatic

The glucosinolates in *Brassica napus* (L.) has been reported in numerus publication, for instance, Gimsing *et al*. (2005) identified eight glucosinolates from Indian

mustard (*Brassica juncea*) and rape (*Brassica napus*) tissues. Tao and He (2004) isolated high concentrations of glucosinolates from mustard seed meals. Moreover, Bhandari *et al*. (2015) found different glucosinolate profiles in different plant parts (seeds, sprouts, mature root, and shoot) of nine *Brassica* species including cauliflower, cabbage, broccoli, radish, baemuchae, pakchoi, Chinese cabbage, leaf mustard, and kal. In *B. napus* (L.), the aliphatic glucosinolates are produced mostly in the seed, while the indole glucosinolates are greatest in the leaf and stem tissues and the major aromatic glucosinolates are found in the root (Clossais-Besnard and Larher 1991; Kirkegaard and Sarwar 1999). Blake-Kalff *et al*. (1998) found that the average of aliphatic, aromatic and Indolyl glucosinolates in young leaves of *B. napus* (L.) were 16, 23 and 61 %, respectively.

Velasco *et al*. (2008) noted that the glucosinolate concentrations in *B. napus* seeds were higher than the leaves. The percentage of aliphatic glucosinolates in seeds was between 91 to 94 %, while there was more variation in the leaves and in the root, with the aliphatic glucosinolates accounting for up to 80% from the total glucosinolate content. Progoitrin was reported to be dominant, whilst the main glucosinolate formed in leaves was glucobrassicanapin. Other experiments were conducted by Fang *et al*. (2012) to determine the glucosinolate content in four different parts ; hypocotyl and radicle, inner cotyledon, outer cotyledon seed coat and endosperm of rapeseed (*B. napus* L.). No variation in concentration of glucosinolates was found between all embryo parts.

Glucosinolate content in *B napus* (L.) leaf was varied between 0.10 to 4.76 μmol. g^{-1} dry matter and dominant glucosinolates found in the leaves were progoitrin, gluconapin and glucobrassicanapin (Cleemput and Becker 2012). Embaby *et al*. (2010) investigated the glucosinolate profile in canola meals from six varieties in Egypt. They identified 12 glucosinolates in all varieties with progoitrin, epiprogoitrin,

gluconapin, glucobrassicanapin and glucoberin being the most abundant aliphatic glucosinolates. The concentrations of indolic glucosinolates 4-hydroxyglucobrassicin, glucobrassicin and 4- methoxybrassicin and aromatic glucosinolate gluconasturtiin were low compared with aliphatic glucosinolates. El-Beltagi and Mohamed (2010) noted there was little variation in the glucosinolate profile among *B. napus* (L.) cultivars; but, gluconapin and progoitrin were the dominant glucosinolate identified, and the highest total glucosinolate content was found in the cultivar Silvo was 5.97 μmol. g^{-1} dry matter.

1.7.1. Glucosinolate biosynthesis

In general, aliphatic glucosinolates are the most commonly produced glucosinolates and are produced from methionine. Glucosinolates come from amino acids and are based on the structure of the amino acid. Glucosinolates are classified into three groups: (i) aliphatic glucosinolates are produced from; isoleucine, leucine, alanine and methionine (ii) aromatic glucosinolates are produced from tryptophan and phenylalanine (iii) indolic glucosinolates are come from tryptophan.

As shown in (Figure 3.2) glucosinolates biosynthesis consists of three main stages; a) control elongation of side chain amino acids (isoleucine, leucine, alanine, methionine and tryptophan), b) modification of amino acids to the core structure of glucosinolates and c) secondary adjustments of the amino acid side chain (Halkier and Gershenzon 2006).

In the first step, chain elongation is stimulated by methylthioalkylmalate (MAM) synthases and condensation of acetyl-CoA. Additional chain elongations can take place by frequent cycles of acetyl-CoA condensation (Verkerk *et al*., 2009).

According to Grubb and Abel (2006) and Mikkelsen *et al*. (2004) synthesis of a core glucosinolate structure is completed in five steps and starts with the oxidation of

amino acids precursor to aldoximes by side chain - specific cytochrome P450 monooxgenases (cytochrome P450) of the CYP79 gene family. The aldoximes are extra oxidised by cytochromes P450 of the CYP83 gene family to aci-nitro compounds or nitrile oxides, which are strong electrophiles that interact naturally with thiols to form S-alkylthiohydroximates. Next, a C-S lyase causes the spilt of the S-alkylthiohydroximate joined into thiohydroximates. Thiohydroximates are reactive and unsteady compounds and those compounds glycosylated and sulphated from glucosinolates core structure.

The final phases of glucosinolates biosynthesis are secondary modifications of the side chain, esterifications, oxidations, eliminations and alkylations. Methionine-derived glucosinolates are formed by two α-ketoglutarate- dependent dioxygenases, encoded by the strongly linked and replicated AOP2 and AOP3 genes which control production of alkenyl and hydroxyalkenyl glucosinolates (Kilebenstein *et al*., 2001; Crubb and Abel, 2006). A flavin monooxygenase that is localized within the GS-OX locus is possibly in authority for the S-oxygenation in the glucosinolate side chain of aliphatic glucosinolates, the indolic glucosinolate glucobrassicin hydroxylation caused by the gene CYP81F2 (Sønderby *et al*., 2010).

Next, in biosynthesis of glucosinolate the MAM, CYP79, CYP83 and AOP gene families, MYB genes were involved. MYB28, MYB29 and MYB76 genes of transcription factors and for production of aliphatic glucosinolate those factors are controlled expression of the structural genes (Sønderby *et al*., 2010).

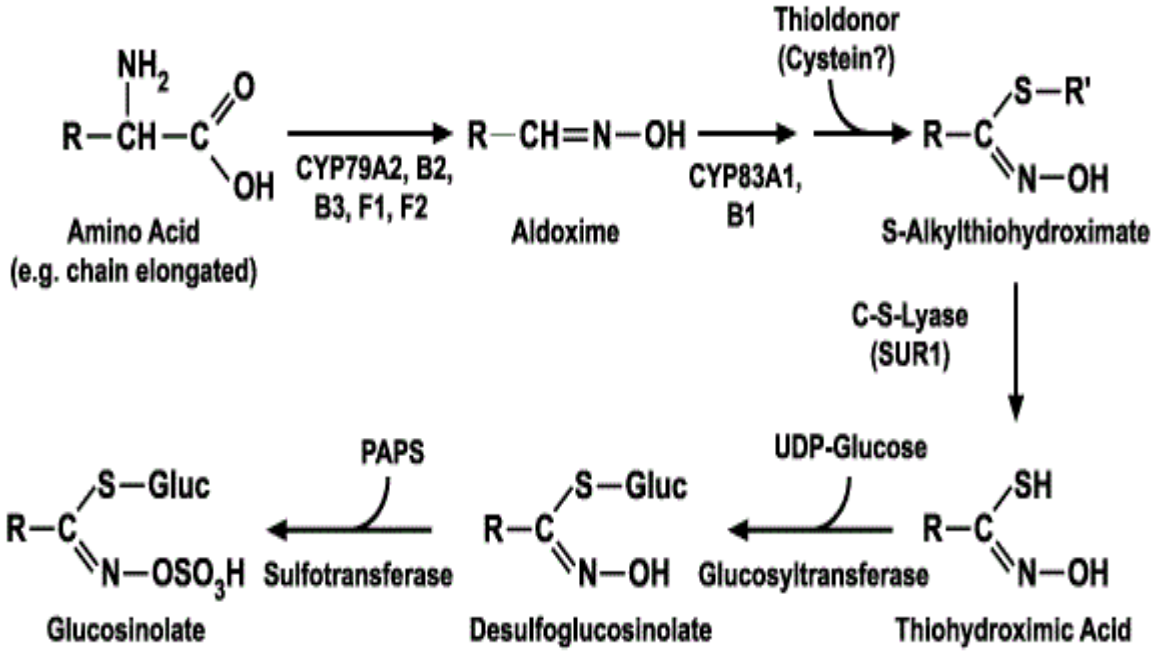

Figure 1. 3. Glucosinolate biosynthesis. (Verkerk *et al*., 2009).

1.7.2. Glucosinolate hydrolysis

Glucosinolates can be hydrolyzed by the myrosinase enzyme (Thioglucoside hydrolase E.C.3.2.1.147) to produce isothiocyanates, nitriles, thiocyanate and epithionitriles. However, the effect of undamaged plant tissue which contains glucosinolates is very little on the other organism (Rask *et al*., 2000).

The myrosinase enzyme is isolated from glucosinolates in undamaged plants by cell organelles. When the plant tissues are damaged during freezing and thawing, grazing, chopping, mastication and wounding or insect and pathogen attack the glucosinolates come into contact with myrosinase (Bennett *et al*., 2006). (Song *et al*., 2005). When the glucosinolates are hydrolyzed, a number of compounds are produced like isothiocyanate, oxazolidinthione or rhodanid, thiocyanate and nitrile (Figure 1.4) which have toxic effects against some organisms (Wittstock *et al*., 2004).

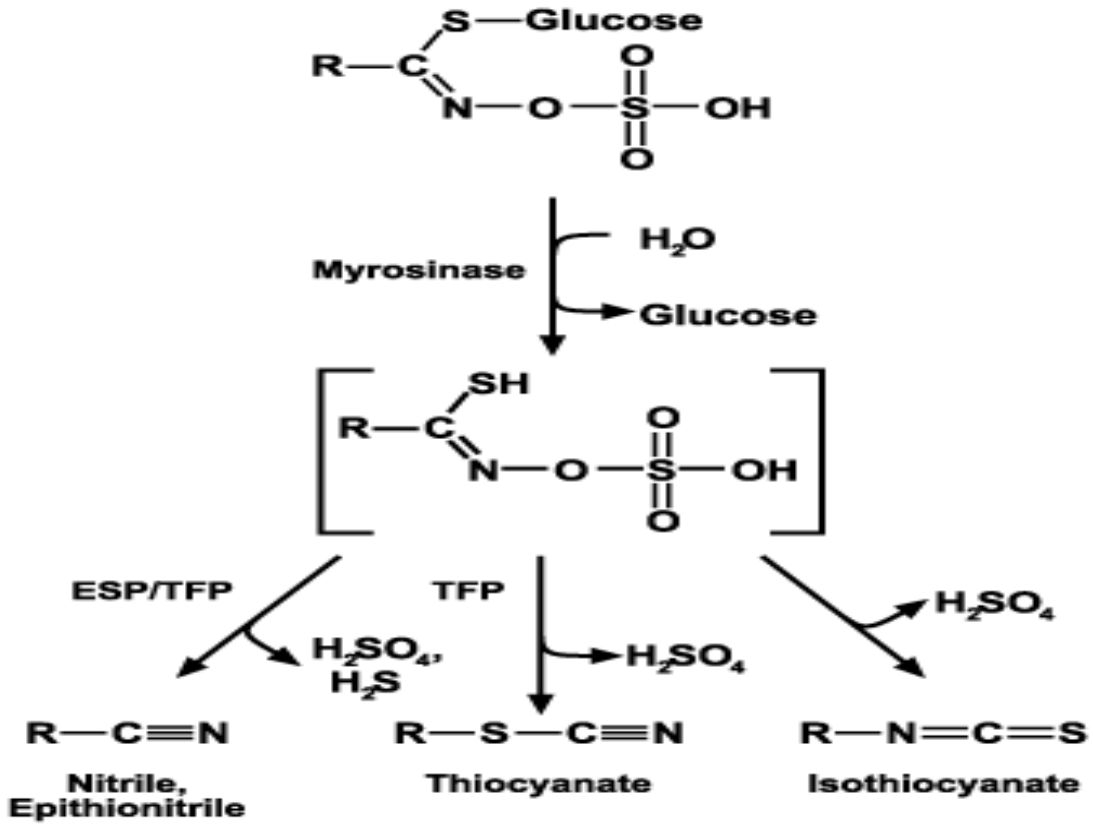

Figure 1.4. Glucosinolate hydrolysis by the enzyme myrosinase. (Fahey *et al*., 2001).

1.8. Myrosinase

Myrosinases (Thioglucoside hydrolase, E.C.3.2.3.1) are enzymes that have the ability to hydrolyse the glucosinolates to produce isothiocyanates, nitriles and thiocyanate. Myrosinases has been found in the *Brassicaceae* and in some fungi and bacteria (Rask *et al*., 2000; Bor *et al*., 2009). In the early eighteen century, Myrosinase was found in *Brassica nigra* seeds by Bussy (1840). Myrosinase activity has been detected in all plants containing glucosinolates (Rask *et al*., 2000; Al-Turki & Dick, 2003). Myrosinase belongs to a family of enzymes which are involved in plant protection against herbivores, the enzyme is a member of glycoside hydrolase family and has a three-dimensional structures as well as numerous similarities with the *O*-glycosidases (Halkier and Gershenzon, 2006; Bones and Rossiter, 2006).

Different forms of myrosinase have been found in many plants and determined as a disulfide-linked dimer of 62-75 kDa subunit (Bones and Slupphaug, 1989; Bones & Rossiter 1996), even though immunological data suggests that they can form complexes of higher molecular weight with myrosinase compulsory proteins and myrosinase linked proteins. In a study conducted by James & Rossiter (1991), several isoenzymes were found in *Sinapsis alba* seeds extracts. These authors found that the enzymatic activity of two isoenzymes in five day old *Brassica napus* seedlings differed depending on the substrate glucosinolate. Below is a three dimensional structure of myrosinase from *Sinapsis alba* seeds (Figure 1.5) (Natarajan *et al*., 2015).

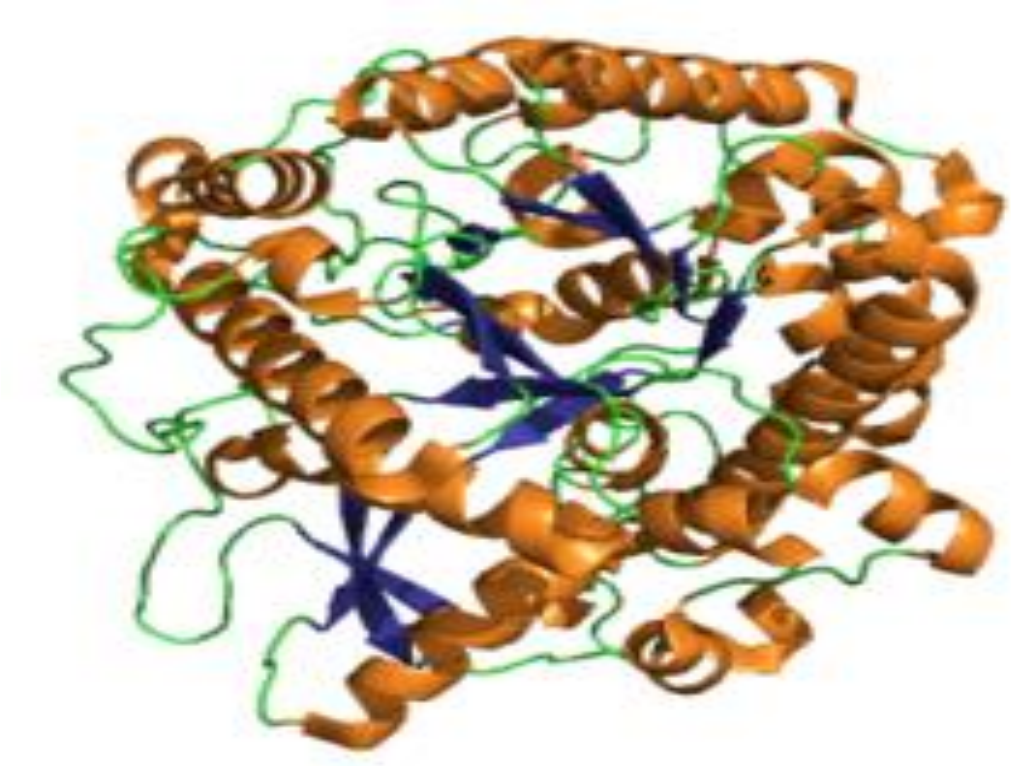

Figure 1.5. Three dimensional structure of myrosinase (Thioglucosidase) from *Sinapis alba* seeds (Natarajan *et al*., 2015).

1.8.1. The myrosin cell

In 1884, the myrosin cell was observed in Brassicaceae species and these cells differed in morphology and size compared with neighbouring cells. The term 'myrosin cell' was used for the first time in 1890, researchers expected that these cells would contain myrosinase within the plant (Rask *et al*., 2000; Halkier and Gershenzon, 2006).

Bones and Rossiter (1996) and Andréasson *et al*. (2001) have identified the myrosin cells in roots, stems, leaves, petioles, seeds and seedlings. The morphology of myrosin cells differs based on both organ and tissue, and age of tissue. Additionally, they found myrosin cells in the phloem parenchyma, while no myrosin cells were revealed in the ground tissue, whereas in *Brassica napus* phloem parenchyma and ground tissue myrosinase could be revealed in myrosin cells.

Bones *et al*., (1991) observed that myrosin cells in *B. napus* are not subject to large developmental changes during the first two weeks after germination. Bones & Iversen (1985) report that the myrosin cells number and myrosinase activity reduced during aging of the plant.

1.8.2. Myrosinase activity

Myrosinase activity is dependent on plant species, cultivar plant region and age and is affected by pH, temperature, ascorbic acid and metal ion, for example Fe^{+2} and Zn^{+2} (Bones and Rossiter, 1996; Osbourn, 1996; Charron *et al*., 2005). Previous work has determined that the greatest levels of myrosinase activity occur in seeds and seedlings. Also, different myrosinase isoenzymes have been recognized in different plant organs of the same plant. No direct correlation between myrosinase activity and glucosinolate levels in plant tissues have yet been detected (Rask et al.

2000). It has been found that myrosinase activity in *S. alba* tissues was approximately ten times higher than *B. campestris*, while the myrosinase activity in *B. napus* was slightly higher as compared with *B. campestris* (Bones, 1990). This author also documented that myrosinase activity in hypocotyls tissues was greatest as compared with other seedling tissues. For example, myrosinase activity in hypocotyls tissues was approximately twice higher than in seedling roots. Overall, the myrosinase activity appears to by greatest in young tissues as compared with the mature tissues of the plant (Bones 1990).

Botti *et al*. (1995) reported that the myrosinase activity was increased by ascorbic acid. In addition, Bones and Slupphaug (1989) observed that the activity of myrosinase increased with low concentrations of ascorbic acid and high concentrations of ascorbic acid inhibited the activity of myrosinase; they also found that in *Brassica napus* the most favorable concentration of ascorbic acid to activate the myrosinase was (0.3-0.5 mM). Increased activity of myrosinase during seed germination (James & Rossiter 1991), may be due to increased ascorbic acid concentration early seedling growth (Sukhija *et al*. 1985).

The activity of myrosinase isoenzymes with low molecular weight (65-58 kDa) increased via ascorbic acid, even though isoenzymes with high molecular weight around (75-77 kDa) were not activated (James & Rossiter 1991; Bones *et al*. 1994). On the other hand, the activity of myrosinase was strongly reduced by the heavy metal ions Cu^{2+}, Fe^{2+}, Fe^{3+} and Zn^{2+} even at lower concentrations (Rai *et al*., 2013). Charron and Sams (2004) reported that the myrosinase activity in fresh leaves was around 30% higher than stems at 12 and 32 °C compared with 22 °C.

In vitro study conducted by Sharma and Garg (1996) to investigate the factors affecting myrosinase activity and enzyme concentration. The authors documented that a pH of 7.0 and a temperature 37˚C were found to be optimum for highest

enzyme activity. During early seedling growth of *Brassica napus* (L.) the myrosinase enzymes were optimally active at pH 5 to pH 6 (James and Rossiter 1991).

In addition it has been suggested that abiotic stress such as salinity, water stress, temperatures and light may enhance the myrosinase activity and its substrate affinity in such a way that the hydrolysis products of the glucosinolates (isothiocyanates) might cause the inhibition of inward K+ channels in the guard cells to decrease water loss by closing stomata (Zhao *et al*., 2008). Guo *et al*. (2013) investigated the effect of different levels of NaCl on glucosinolates concentration and myrosinase activity in broccoli. The data showed that higher content of glucosinolates in broccoli was observed by NaCl treatment at the concentration of 60 mmol/L for 5 days old. However, the activity of myrosinase decreased.

1.9. Herbicidal potential of glucosinolate

With tightening legislation on pesticide usage, there is increasing interest in alternative method of crop protection such as biofumigation. Brassica species contain glucosinolates may have ability to suppress seed germination and seedling growth, and may also inhibit a number of insect species and fungi (Brown and Morra, 1996; Brown and Morra, 1997 Borek *et al*., 1998; Sarwar and Kirkegaard 1998)

The growth of competing vegetation has been suppressed by a number of Brassicaceae plants, and the hydrolysis products from numerous glucosinolates have been isolated and shown to inhibit both plant or seed germination (Rosa *et al*. 1997).

Plant species containing glucosinolates have a reputation for suppressing the growth of neighboring weeds and crops such as wild oat (*Avena sterilis*), wheat and pea (Jones, 1992). Vaughn *et al*. (2006) observed a significant reduction in the wheat seedling emergence with using seed meals from 15 glucosinolate-containing

plant species at 1% (w/w) concentration with sandy loam soil. Seed meals from Indian mustard, money plant, and field pennycress completely inhibited wheat seedling emergence. The seedling emergence inhibition may be due to both the type and concentration of glucosinolates and their hydrolysis products in the seed meals. These authors identified several glucosinolates; glucoerucin, glucotrapaeolin, glucoiberin, gluconapin, and glucoraphenin in the seed meals of 15 different plant species and showed that isothiocyanates produced from these glucosinolates were able to inhibit the germination of sicklepod (*Senna obtusifolia*).

Brown and Morra (1996) reported that glucosinolate hydrolysis products from *Brassica napus* tissues, especially leaf and stem, can inhibit the seed germination of *Lactuca sativa* and these findings may support the suggestion that the plant tissues containing glucosinolate may help to decrease the use of synthetic herbicides for weed control. Moreover, Arslan *et al*. (2005) conducted an experiment to identify the effect of bio-herbicidal of fresh shoot and root extracts of six *Brassica species* by different concentrations on cutleaf ground-cherry (*Physalis angulata* L.) seed germination.

A glasshouse experiment was conducted by Norsworthy *et al*. (2005) to determine the herbicidal activity of five aliphatic and three aromatic isothiocyanates on three weed species. The isothiocyanates were applied in different concentrations to soil at 0, 10, 100, 1,000 and 10,000 nmol g^{-1} of soil and incorporated. All isothiocyanates had a harmful effect on Palmer amaranth and pitted morningglory emergence. The most effective isothiocyanates against yellow nutsedge was Phenyl and 3-methylthiopropyl at 10,000 nmol g^{-1} of soil and the emergence reduced by 92%.

1.10. Rational for this study

The main field crop in Iraqi Kurdistan rain-fed area are cereal crops (Ismail, 2006; Marof 2007), crops yield was reduced approximately by 45 % because of weed plants (Al- Ali, 1982). In Kurdistan, cotton and lentil yields were found to be reduced by 65% and 35% respectively due to weed growth (Sultan and Aliki, 2003; Aliki *et al*., 2006). Little work has been done on allelopathic potential of crop plant species on weed species such as durum wheat *Triticum durum*, Barley *Hordeum vulgare* and Oat *Avena sativa* (Ali *et al*., 2012; Ali, 2013). For example, Ali *et al*. (2012) found that effect of using wheat straw water extracts significantly reduced plants height, levels of chlorophyll a, b and total chlorophyll of wild oat *Avena fatua*, canary grass *Phalaris minor* and cow cockle *Vaccaria pyramidata*. However, no literature available about using allelopathic potential of *Brassica* species on weed species.

In addition, in Iraq and specifically the Kurdistan Region, due to economic blockades and wars for four decades there has been lack of investment in agriculture, agricultural extension and educating farmers on the safe use of pesticides. These factors have contributed to poor use of pesticides and the lowering of yields. More alternative strategies and must be investigated for sustainable weed management.

1.11. Conclusion

Allelopathic potential of *Brassica spp*. for weed suppression has been focused on the employment of *Brassica spp*. as natural herbicides to control weed species as discussed above.

From the above review it seems that allelopathy can be used in several aspects of crop production. One of the main applications of allelopathy in crop production is for weed control. Various studies have been applied to explore the probability of using allelopathic crops to suppress weed germination and seedling growth in

agricultural sites. Crop plants have the ability to produce and exude allelochemicals in to the environments from different plant parts and during different growth stages to inhibit the growth of weeds in their neighborhood. Intercropping of allelopathic crops may improve the productivity of mixtures on one side and sustainable weed suppression on the other.

Brassica napus is a new crop in the Kurdistan region of Iraq with unique characteristics that may provide new opportunities for farmers that are being encouraged to plant a cover crop to provide environmental benefits. *Brassica napus* can provide a variety of benefits to cropping systems, including weed inhibition. *Brassica napus* water extracts can inhibit weeds by several of mechanisms. The mechanisms of weed suppression by *Brassica napus* water extracts are not currently understood. Research is needed to evaluate the repeatability and duration of *Brassica napus* weed inhibition and to identify the mechanisms of this weed inhibition in order to develop management practices to best take advantage of this weed inhibition in the Kurdistan region.

Furthermore, GSL biosynthesis and accumulation differs among *Brassica spp* parts and can be affected by many environmental factors such as water stress, light and temperature and also plant development stages. Because GSL biosynthetic regulation and accumulation vary between different *B. napus* parts tissues, *B. napus* provides a uniquely well-suited crop to examine the differential effect of environmental factors on root, stem, flower and leaf GSL concentrations and myrosinase activity. Better understanding of the affect of the environmental factors on GLS concentrations and myrosinase activity in different parts of *B. napus* will provide useful information to maximize the inhibitor effect on weed species.

1.12. The objectives and Null hypotheses

The main objective of this study is to develop an understanding of the allelopathic properties of *Brassica napus* (L.) plants in order to improve methods for controlling different weeds species *Sorghum halepanses* (L.) (Johnsongrass), *Convolvulus arvensis* (L.) (field bindweed), and *Phalaris minor* (Retz.) (canary grass) in laboratory. These three weed species were chosen because of their high spread in fields and subsequent cause of large losses in the yield of farmers.

The specific objectives of the present study are:

- Identify allelopathic weed suppression from *Brassica napus* (L.).
- Elucidate allelopathic chemicals responsible from *Brassica napus* (L.) for weed suppression.
- Investigate the effects of water extract concentrations from different parts of *Brassica napus* on seed germination and seedling growth of weeds species.
- Assess the allelopathic chemicals produced by *Brassica napus* (L.) during different stages of growth and their influence on weed species inhibition.
- Assess the levels of glucosinolates and myrosinase enzyme activity produced by *Brassica napus* (L.) from different parts under water stress conditions and their influence on weed species inhibition.
- Assess the effect of using pure glucosinolates on seed germination and growth of wees species tested.

The hypothesise studied were

- Water extracts applications from *Brassica napus* will not affect the level of weed germination and seedling growth.

- There is no significant differences between using water extract from different parts of *Brassica napus* on weed germination and seedling growth
- Water extract concentrations from different parts of *Brassica napus* will not affect seed germination and seedling growth of weeds species.
- Water stress levels during different plant growth stages will not influence the levels of glucosinolates and myrosinase enzyme activity produced by *Brassica napus* (L.) from different parts and their effect on weed species.
- There is no significant differences between the levels of glucosinolates and myrosinase enzyme activity produced by *Brassica napus* (L.) from different parts during different growth stage and their effect on seed germination and seedling growth of weed species tested.
- Pure glucosinolates will not affect seed germination and seedling growth of weed species tested.

Chapter 2

2. The use of allelopathic plant species to manage weed species in Iraq

2.1. Introduction

Allelopathy is defined as the beneficial or harmful effect of one plant on another plant due to the production of certain chemical compounds (Naseem *et al*., 2009). Allelopathy has been proved to have the ability to control pests, weeds, insects, nematodes and pathogens (Jabran *et al*., 2008; Niknechan *et al*., 2011; Farroq *et al*., 2013). There are numerous reports that some crop plants have allelopathic effects on seed germination and seedlings growth of weed species (Rice, 1984; Shibu and Andrew, 1998; Delabays *et al*., 2004; Mulatu *et al*., 2009).

The toxic effects of water extract from different parts tissue of crops have been reported by many researchers. According to Putnam (1988) the chemical substance with allelopathic potential present in all plants part tissues, like leaf, stem, flower, fruit, seed and root. Under different conditions, these allelochemicals are released in to atmosphere or rhizosphere in high quantities and long persistence to affect a neighbouring plant. Several brassica species have significant effects on seed germination and seedling growth of weeds due to chemical compounds found in brassica water extract include caffeic, ferulic, chlorogenic and vanillic acids, thiocyanates and isothiocyanates (Bell and Muller, 1973; Al-Saadawi *et al*., 1986; Putnam, 1988; Vaughn and Berhow, 1999; Widmer and Laurent, 2006; Haddadchi and Gerivani, 2009).

The water extracts of brassica species showed phytotoxicity to various weed species. For instance, seed germination and seedling growth of wild oat, alfalfa, lentil and wild radish were inhibited by *Brassica nigra* L. water extracts from different parts such as leaf, root, stem and flower (Turk & Tawaha, 2002; 2003, Turk et al., 2003; Turk et al., 2005). Growth of (Zea mays) (Zaji & Majd, 2011), soybean (Oskoeui et al., 2012) and different weeds (Uremis et al., 2009) were suppressed

by water extracts of *B. napus*. From different parts. Ina addition , seed germination of cutleaf ground-cherry (*Physalis angulate* L.) inhibited by *B. compestris* water extract from shoot and *B. rapa* root water extract by 58.7%and 54.3% respectively (Arslan *et al*., 2005). Also. In a greenhouse trials, the biomass of hairy nightshade and long spine sandbur was reduced by 90 and 83% when rapeseed tissue added to a sandy soil (Boydston and Hang, 1995).

Although, some work has been carried out on the allelopathic potential of crops in weed control in the Kurdistan Region of Iraq, there has been lack of investment in agriculture, agricultural extension and educating farmers on the safe use of pesticides due to wars and economic blockade for the last three decades. These factors have contributed to the poor use of pesticides and reduced yields. The purpose of the experiments presented in this chapter were to determine the effect of *Brassica napus* (L.) water extracts from different parts of *B. napus* plants on germination and seedling growth of weed species found in Iraq.

2.1.1. Aim

The aim of this experiment was to determine the effect of *B. napus* water extracts from different plant parts on weed species germination and seedling growth.

2.1.2. Hypotheses

i- The level of weed germination and seedling growth will not be affected by water extracts applications from *Brassica napus*.

ii- There are no significant differences between using water extract from different parts of *Brassica napus* on weed germination and seedling growth.

2.2. Materials and methods

2.2.1. Preparation of water extracts

Oilseed rape (*Brassica napus* L.) (cv. PR46W21) plants were collected from First Fox Hole field at Harper Adams University (Edgmond, Newport, Shropshire, England, UK) at the beginning of flowering (GS 4.5). Fresh rapeseed plants were separated into leaves, stems, roots and flowers. leaves, stems , roots and whole plants cut into 1 cm pieces and fresh tissue (100 g kg^{-1}) from each plant part and whole plants were soaked separately in 1 L distilled water (1:10 W/V) for 24 hours at room temperature (20 ^{0}C± 2) to obtain water extracts (Turk and Tawaha, 2003). This solution was filtered through two sheets of filter paper (Whatman No.2) to remove the solid organic material and stored in a freezer (-25 ^{0}C± 2) until required.

2.2.2. Bioassay

Three weed species *Sorghum halepanses* (L.) (Johnsongrass), *Convolvulus arvensis* (L.) (field bindweed), and *Phalaris minor* (Retz.) (canary grass) were purchased from Herbiseed Ltd, Berkshure, UK. Seeds (250) from each weed species were surface sterilized by soaking them in 5% sodium hypochlorite for 15 minutes before twice washing them in distilled water. Twenty seeds from each weed species were evenly placed on filter paper (Whatman No.1) in 9 cm petri dishes. Ten ml of extract solution from each plant part (stem, leaf, flower, root and whole plant) were added to each petri dish and distilled water was used as a control. All Petri dishes were placed in plant growth chambers (Sanyo MLR) randomly at 25°C and 70% humidity and in continuous darkness. Treatments were arranged in a completely randomized design (CRD) with five replications (6 Plant water extract types x 5 replications). The experiment was repeated three times to ensure reliability of results.

2.2.3. Assessment

Germination percentage was calculated for fourteen days after sowing using the equation (2.1) (ISTA, 1976).

$$\text{Germination}(\%) = \frac{\text{Number of seeds germinated}}{\text{Number of total number of seeds}} \times 100 \qquad (2.1)$$

Shoot and root length was measured (cm) for all seedlings at fourteen days using a caliper. Shoot and root fresh weight (g) was measured by using electronic balance (Precisa 262 SMA- FR) in the same seedlings that were used for shoot and root length.

2.2.4. Experimental design and data analysis

A completely randomized design (CRD) with five replications (6 Plant water extract types x 5 replications) for each weed species was used for the experiment. Data of each weed species were used separately for statistical analysis using Kruskal-Wallis One-Way Analysis of Variance (nonparametric test) using Genstat 14th Edition (Release PL21.1, Lawes Agricultural Trust, Rothamsted, UK), because there was no germination in some treatments Differences between the means were tested by Kolmogorov-Smirnov two sample tests. The full outputs of Kolmogorov-Smirnov two sample tests are displayed in the Appendix (1).

2.3. Results

2.3.1. Seed germination %

Water extracts from different parts of *Brassica napus* plants showed a significant (*P*<0.001) inhibitory effect on the seed germination of *Phalaris minor* (Retz.), *Convolvulus arvensis* (L.) and *Sorghum halepense* (L.). Water extracts from flowers and leaves caused the lowest germination when compared with the control (Figure 2.1). The Kolmogorov-Smirnov two sample test showed that there were also significant differences between the treatments themselves. Water extracts from leaves, flowers, stems and roots were found be more effective on *P. minor*(Retz.), *C. arvensis* (L.) and *S. halepense* (L.) when compared with water extract prepared from the whole plant and with the control (Figure 2.1, Appendix 1).

2.3.2. Shoot length (cm)

The effect of *B. napus* water extract treatments on shoot length was highly significant (*P*<0.001). Shoot length (cm) of *Phalaris minor* (Retz.), *Convolvulus arvensis* (L.) and *Sorghum halepense* (L.) was significantly (*P*=0.007) reduced by using water extract from all parts of *B. napus* (L.).

There was a significant difference between water extract treatments. Flower water extract had a significant (*P*=0.007) effect on shoot length of all three species of weeds. Water extracts from flowers and leaves decreased shoot length of *P. minor* (Retz.) by 100% and 50.1% respectively. Flower and stem water extracts were found to inhibit the shoot length of *C. arvensis* (L.) by 89.3% and 37.6% respectively. Also, the shoot length of *S. halepense* (L.) was reduced by flower and stem water extract up to 83.5%and 91.9% respectively (Figure 2.2, Appendix 2).

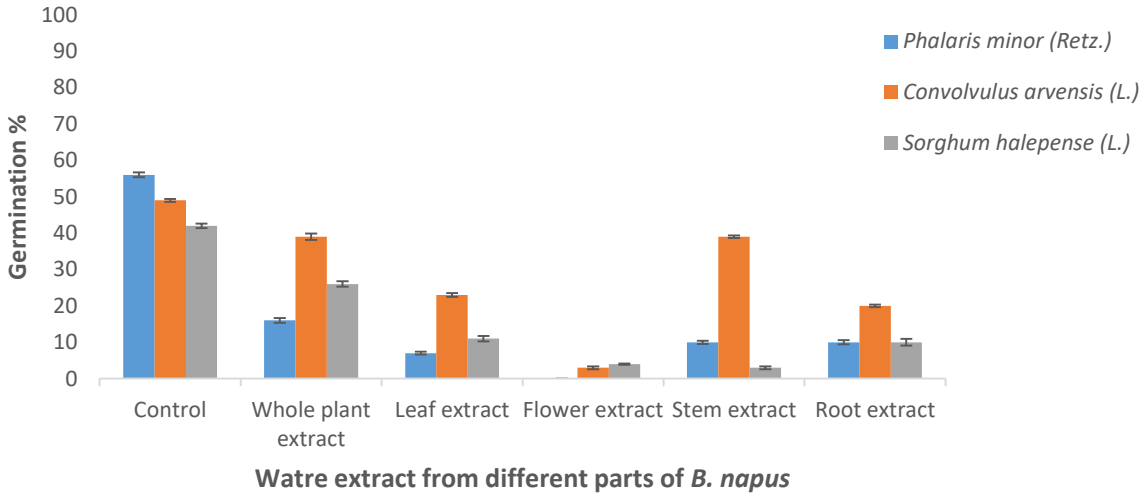

Figure 2.1. Effect of various *Brassica napus* (L.) water extracts on the seed germination % of *Phalaris minor* (Retz.), *Convolvulus arvensis* (L.) and *Sorghum halepense* (L.) 14 days after sowing. n = 25 for each treatment. Error bars represent the standard error of the mean.

2.3.3. Shoot weight (mg)

Brassica napus (L.) water extract from all parts had a significant (*P*<0.001) effect on shoot weight (mg) of *Phalaris minor* (Retz.), *Convolvulus arvensis* (L.) and *Sorghum halepense* (L.). There were significant differences between the treatments with exception of the whole plant water extract. Flower water extract had the most significant (*P*=0.007) effect on weight (g) of weeds, especially *C. arvensis* (L.). Water extract from flowers and leaves decreased shoot weight of *P. minor* (Retz.) by 100% and 69.2% respectively. Meanwhile, leaf, stem and root extracts were also effective on *C. arvensis* (L.) and reduced shoot weight by 47% to 48% compared to the control. Also, the shoot weight of *S. halepense* (L.) was reduced by flower and stem water extract by up to 97.5% and 95.6% respectively (Figure 2.3, Appendix 3).

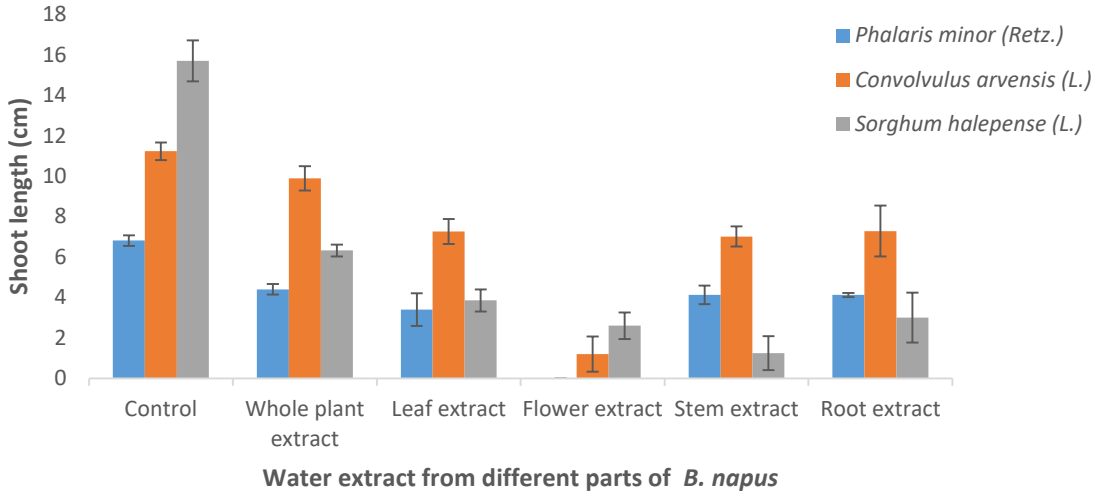

Figure 2.2. Effect of various *Brassica napus* (L.) water extracts on the shoot length (cm) of *Phalaris minor* (Retz.), *Convolvulus arvensis* (L.) and *Sorghum halepense* (L.) 14 days after sowing. n = 25 for each treatment. Error bars represent the standard error of the mean.

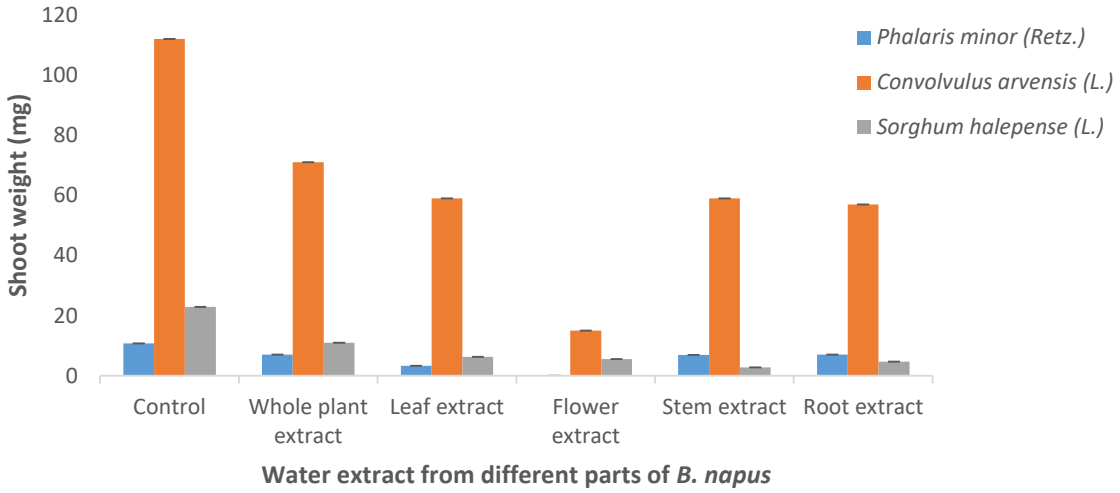

Figure 2.3. Effect of various *Brassica napus* (L.) water extracts on the shoot weight (mg) of *Phalaris minor* (Retz.), *Convolvulus arvensis* (L.) and *Sorghum halepense* (L.) 14 days after sowing. n = 25 for each treatment. Error bars represent the standard error of the mean.

2.3.4. Root length (cm)

The root length of *Phalaris minor* (Retz.), *Convolvulus arvensis* (L.) and *Sorghum halepense* (L.) were affected significantly *(P*<0.001) by using all *B. napus* (L.) water extract treatments compared with control. There was a significant difference between water extract treatments themselves. Water extracts from leaves, roots and (particularly) flowers produced a significantly higher (*P*=0.007) effect on the root length of all three weed species. Flower and root water extract showed higher reduction in *P. minor* (Retz.) and *C. arvensis* (L.) root length up to 100%, 77.9% and 100%, 82.3% respectively. Leaf, flower and root extracts decreased *S. halepense* (L.) root length by 100% compared to the control (Figure 2.4, Appendix 4).

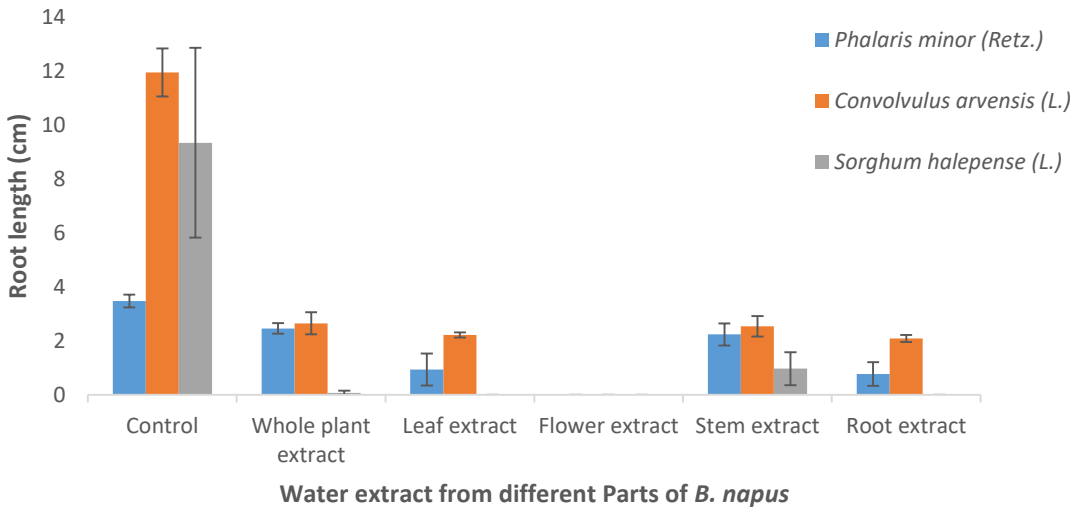

Figure 2.4. Effect of various *Brassica napus* (L.) water extracts on the root length (cm) of *Phalaris minor* (Retz.), *Convolvulus arvensis* (L.) and *Sorghum halepense* (L.) 14 days after sowing. n = 25 for each treatment. Error bars represent the standard error of the mean.

2.3.5. Root weight (mg)

It was found that the root weight of *Phalaris minor* (Retz.), *Convolvulus arvensis* (L.) and *Sorghum halepense* (L.) significantly (P<0.001) decreased with the different treatments. Kolmogorov-Smirnov two sample tests determined that treatments of whole plant, leaf, flower, stem and root water extracts significantly reduced the root weight (mg). Moreover, root weight of *P.s minor* (Retz.) was suppressed by flowers and leaves water extract up to 100% and 81.8% respectively. Flower extract reduced the root weight of *C. arvensis* (L.) by up to 100% Also, flower, leaf and root extract inhibited the *S. halepense* (L.) roots weight up to 100%. The results in (Figure 2.5, Appendix 5), show that *Phalaris minor* (Retz.) and *Sorghum halepense* (L.) were most affected by water extract from all plant parts.

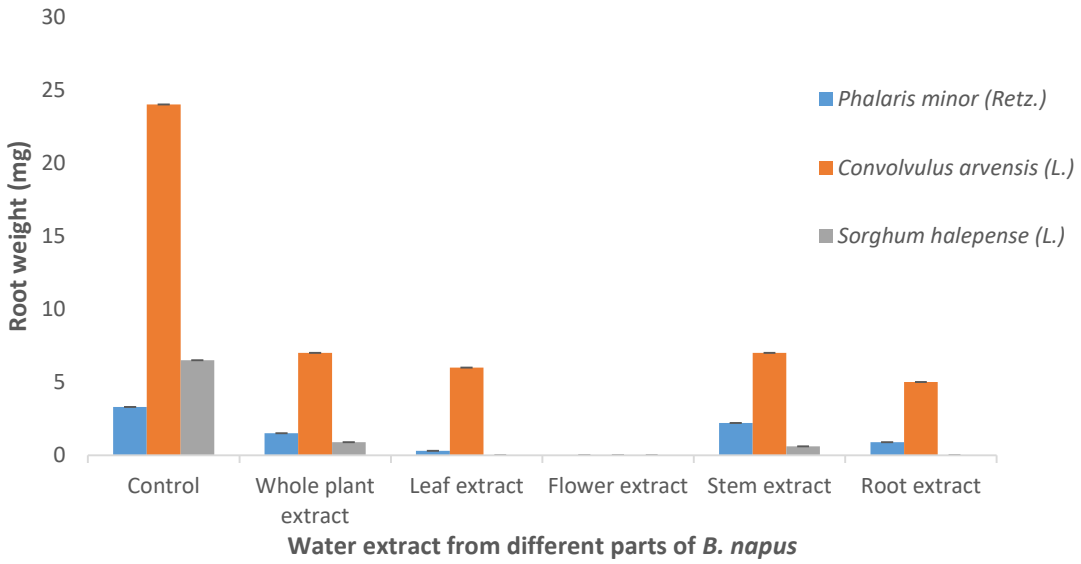

Figure 2.5. Effect of various *Brassica napus* (L.) water extracts on the root weight (mg) of *Phalaris minor* (Retz.), *Convolvulus arvensis* (L.) and *Sorghum halepense* (L.) 14 days after sowing. n = 25 for each treatmentError bars represent the standard error of the mean.

2.4. Discussion

The present study attempts to evaluate the ability of *B. napus* (L.) water extracts to inhibit germination and seedling growth of three weeds species that are important in Kurdistan region. The results of the present study have indicated that extracts of *B. napus* (L.) have a suppressive effect on the germination of *P. minor* (Retz.), *C. arvensis* (L.) and *S. halepense* (L.). Although all extract treatments had some effect on germination of weed species, flower water extracts were the most effective on inhibiting the germination of the weed species tested. Using flower extracts there was no germination in *P. minor* (Retz.), *C. arvensis* (L.) and *S. halepense* (L.) germination was reduced by 93.88% and 90.5% respectively. A similar result was reported by Turk and Tawaha (2003) who found that *Brassica nigra* (L.) water extracts from different plant parts (lea, stem, flower and root) reduced wild barley germination, dry weight, shoot length and root length compared with the control. This may be due to the presence of phenolic compounds which inhibit the activity of gibberellic acid and also inhibit the cell division and elongation process that are very important at this stage or by interfering with enzymes involved in the mobilization of nutrients necessary for germination (Einhellig, 1996; Levizou *et al*, 2002). Yukiko *et al*. (2001) reported that phenolic compounds had the ability to reduce the seed germination of shirakamba birch (*Betula platyphylla* Sukatchev var.) by 60% to 100%. Peterson *et al* (2001) found that isothiocyanates are one of the important chemical compound in *Brassicas* that have the ability to inhibit the germination of many weeds species such as *Sonchus asper* L. Hill), scentless mayweed (*Matricaria inodora* L.), smooth pigweed (*Amaranthus hybridus* L.), barnyardgrass (*Echinochloa crusgalli* L. Beauv.) and blackgrass (*Alopecurus myosuroides* Huds.).

The results of this study showed that weed species seedlings growth (shoot length, root length, shoot weight and root weight) were inhibited by water extract from all

parts of *Brassica napus* (L.). Shoot and root length of *Phalaris minor* (Retz.), *Convolvulus arvensis* (L.) and *Sorghum halepense* (L.) were affected significantly by all water extract treatments compared to the control. However, the whole plant water extracts were the least effective treatment on weed seedling growth compared to other treatments.

These results are similar with other studies reporting that *Brassica spp* water extracts had an effect on the seedling growth of weeds. Cheema *at el.* (2003) found that brassicas have the ability to reduce density, shoot length, root length, shoot and root weight of weeds such as canarygrass (*Phalaris minor* Retz), wild oat (*Avena fatua* L.), broad leaf dock (*Rumax dentatus* L.), lambsquarters (*Chenopodium album* L.) and field bind weed (*C. arvensis* L.) by 40-50%.

Turk and Tawaha (2003) recorded that water extract from different parts (leaf, stem, flower and root) of black mustard (*Brassica nigara* L.) had strong inhibitory effect on germination and seedling growth of wild oat. They found that the degree to which germination and root length was inhibited by this extract increased with increasing the concentration of the water extract. Also, these authors determined that seed germination of wild oat was reduced and this may be because of the interruption in water uptake may cause a reduction in seed protease activity, which have ability to affect the protein hydrolysis during germination therefore may reduce the imbibition and water uptake of seeds. The findings are agree with the results of (Rice, 1984). Moreover, Babar *et al*. (2009) reported that the germination of chickpea seeds when soaked in root extract of *Asphodelus tenuifolius* were delay compared with the control.

The coefficient of variation (C.V. %) for seed germination, shoot and root length, shoot and root weight was very high and this may be due to the high difference

between the mean values and the data is a non-normal distribution, lower C.V.% can obtain by increseing the number of replications (John, 2009).

2.5. Conclusion

In this experiment water extracts made from different parts of *B. napus* (L.) were used to evaluate their effect on weed species in control conditions. All treatments showed significant inhibition of germination, shoot and root length and shoot and root weight for all weed species compared to the control. The greatest effect was seen with the flower extract especially on *P. minor* (Retz.) and *C. arvensis* (L.), but the stem extract had the greatest effect on *S. halepense* (L.). These findings will help us to develop our knowledge about the effect of *B. napus* (L.) on weed species in further work.

Chapter 3

3. Effects of concentrations of *Brassica napus* (L.) water extracts on the germination and growth of weed species

3.1. Introduction

Allelopathic crops can decrease noxious weeds, prevent plant disease and improve soil quality and crop yield when used as cover crops, mulch, green manures, or grown in rotation (Cheema and Khaliq. 2000; Awan *at el*., 2009; Khalil *et al*., 2010). However, this ability to suppress and control weeds depends on the concentration of the chemical compounds in the allelopathic plant (Bhowmik and Inderjiit, 2003). Various studies revealed that allelochemicals, at high concentrations may be more effective; however, at lower concentrations these allelochemicals may stimulate the growth of other species (Narwal, 1994).

For example, Tosi and Baki (2012) state that *Brassica juncea* leaves ethanol extract at all concentrations 10.8, 14.28, 18 and 30 gL^{-1} inhibited the germination of radish (*Raphanus sativus* L.) seeds. Also, root and shoot growths of barnyard grass and radish seedlings were significantly affected by the leaf stem and root extracts.

Turk and Tawaha (2002) found that the lentil germination and seedling growth decreased by increasing the concentration of water extracts from different parts of black mustard (leaves, flowers, roots and mixture). In additional work Afridi *et al*. (2014) investigate the allelopathic potential of different concentrations (0, 50 and 100%) of rice straw extract on germination and seedling growth of different plants. The authors observed that 100% rice straw extract significantly inhibited the germination and growth of *Ipomoea batatas*, *Rumex dentatus* and *Convolvulus arvensis*.

Additionally, Turk *et al*. (2005) evaluated allelopathic effects of black mustard from different plant parts (leaf, stem, flower and root) on radish (*Raphanus sativus*) germination and seedling growth. They found that radish seed germination and seedling growth were inhibited significantly by all water extracts and increasing the water extract concentrations of individual plant parts significantly inhibited seed

germination. Also, germination and seedling growth of quack grass (*Agropyrum repens*) significantly reduced by barley extracts from different parts (leaf, stem, flower and root). The inhibition of *Agropyrum repens* germination, seedling length and weight increased significantly by increasing the water extract concentrations from 4 to 20 g per 100 ml of water (Ashrafi *et al*., 2009).

3.1.1. Aim

The aim of these experiments was to investigate the effect of water extract concentrations from different parts of *B. napus* germination and seedling growth.

3.1.2. Hypotheses

Water extract from different parts of *B. napus* at different concentrations will not affect germination and seedling growth of weeds species.

3.2. Materials and Methods

3.2.1. Experiment 1:

Oilseed rape (*Brassica napus L.*) cv. PR46W21 plants were collected from a field at Harper Adams University, Edgmond, Newport, Shropshire, England, UK at the beginning of flowering (GS 4.5) in March 2012. The field had been treated with a number of pesticides (table 3.1). The same procedure was used to prepare the water extracts from different plant parts (stem, leaf, flower and root) as described in (Chapter 2 section 2.2.1). The water extracts from different plant parts diluted to prepare different concentrations (25%, 50%, 75% and 100% v/v) with sterile distilled water and samples were stored in a freezer (-25 ^{o}C± 2) until required.

Experiment 2:

Oilseed rape (*Brassica napus L.*) cv. PR46W21 was cultivated in the polytunnel at Harper Adams University, Edgmond, Newport, Shropshire, England, UK. Oilseed

rape plants were harvested at the beginning of flowering (GS4.5) in August 2012.The same procedure as experiment 1 was used except preparing different concentrations (3.125%, 6.25%, 12.5%, 25% v/v) with sterile distilled water and samples were stored in a freezer (-25 ^{o}C± 2) until required.

3.2.1.1. Bioassay

This chapter reports the findings of two replicated experiments. Seeds (250) of test weeds [*Phalaris minor* (Retz.) (canary grass), *Convolvulus arvensis* (L.) (field bindweed) and *Sorghum halepanses* (L.) (Johnsongrass)] were prepared as previously described (Chapter 2, section 2.2.2).Ten ml of extract solution from each concentration was added to each petri dish and distilled water was used as control. All Petri dishes were placed in a plant growth chamber (Sanyo MLR) at 25°C in dark condition. Treatments were arranged in a completely randomized design (CRD) with six replications (5 concentratios x 6 replications) for each weed species.

3.2.1.2. Observations

Germination, shoot and root length and fresh weight of seedlings was measured as as previously described (Chapter 2, section 2.2.3).

3.2.2. Statistical analysis

A completely randomized design (CRD) with six replications (5 concentratios x 6 replications) for each weed species was used for the experiment 1 and 2. The data from experiment 1 were not normally distributed because there was no germination and no root growth in some treatments. Consequently, the data was then analysed using a Kruskal- Wallis one -way Analysis of Variance using Genstat 14th Edition (Release PL21.1, Lawes Agricultural Trust, Rothamsted, UK) for each weed species

separately. Differences between the means were tested by Kolmogorov-Smirnov two-sample tests. Data were subjected to a general analyses of variance (ANOVA) one-way to analysis the experiment 2 results for each weed species individually and where necessary, data were log10-transformed to normalise residuals. A Duncan test was used at $P < 0.05$ for comparison of data obtained from different plant parts at concentrations.

Table 3.1. (Pesticides applied to the *Brassica napus* field)

Description	**Field Rate Dosage ha^{-1}**	**Chemical type**	**Date applied**
Centium 360 CS clomazone	0.20 Litres	herbicide	29/08/2011
Oryx metazachlor+ quinmerac	2.02 Litres	herbicide	29/08/2011
Attract	4.04 Litres	herbicide	09/09/2012
Permasect C cypermethrin	0.25 Litres	insecticide	28/10/2011
Fusilade Max fluazifop-P-butyl	0.60 Litres	herbicide	28/10/2011
Harvesan carbendazim+ flusilazole	0.61 Litres	fungicide	28/10/2011
Corinth tebuconazole prothioconazole	0.50 Litres	fungicide	02/03/2012
Headland Boson	2.50	micronutrients	02/03/2012
Delsene 50 Flo carbendazim	0.50 Litres	fungicide	02/03/2012
Toppel 100 EC cypermethrin	0.25 Litres	insecticide	30/03/2012
Priori xtra azoxystrobin+ cyproconazole	0.80 Litres	fungicide	30/03/2012

3.3. Results

3.3.1. Experiment one

3.3.1.1. Seed germination %

The flower, stem, leaf and root extracts of *Brassica napus* significantly (p<0.001) inhibited the seed germination of test weeds [*Phalaris minor* (Retz.), *Convolvulus arvensis* (L.) and *Sorghum halepense* (L.)]. The degree of suppression increased with increasing extract concentrations from different parts (Table 3.2). Kolmogorov-Smirnov two-sample tests showed that there were also significant differences between the treatments (P < 0.05). Water extracts from flowers and stems at 75% and 100% concentrations were more inhibitory to weed species. Undiluted extracts of flowers at 75% and 100% concentrations inhibited the germination of *P. minor* by 87%, 91.4%, *C. arvensis* by 73.3%, 83% and *S. halepense* by 79.75%, 84.9 %, respectively. Also the undiluted extracts of stems inhibited the germination of *P. minor, C. arvensis* and *S. halepense* by 100%%, 95.05 and 99.4% at 100% concentration, respectively, and 99.4%, 81.5% and 94 % at a concentration of 75%, respectively.

3.3.1.2. Shoot length (cm)

Shoot length of *P. minor, C. arvensis* and *S. halepense* was significantly reduced (P< 0.001) by flower, stem, leaf and root extracts at all concentrations in both experiments (Table 3.3). Based on Kolmogorov-Smirnov two-sample tests the extracts from different parts of *B. napus* at various concentrations showed significant differences between the treatments (P < 0.05). All concentrations inhibited the shoot length, and the effect was concentrations dependent. Undiluted flower, stem, leaf and root extracts reduced the shoot length of *P. minor* by 58%, 100%, 61% and 43%, respectively. The same treatments (undiluted flower, stem, leaf and root

extracts), also decreased the shoot length of *C. arvensis* by 45.3%, 82.5%, 73.5% and 44.9%, respectively and reduced the shoot length of *S. halepense* by 57.3%, 96.5%, 77.9% and 65.2%, respectively.

3.3.1.3. Root length (cm)

Brassica napus water extracts from all parts significantly ($P < 0.001$) inhibited the root length of *P. minor, C. arvensis* and *S. halepense*. The sensitivity of each weeds root length to water extract was increased with increasing concentrations (Table 3.4). The *B. napus* flower, stem, leaf and root extracts at 75% and 100% concentrations were more inhibitory to root elongation of *S. halepense* than to *P. minor*. Stem extracts at 75% and 100% concentrations completely inhibited the root length of *P. minor* and *S. halepense*, however, *C. arvensis* root length was reduced by 84.6% and 100%, respectively.

3.3.1.4. Shoot fresh weight (mg)

The fresh shoot weight of *P. minor, C. arvensis* and *S. halepense* was inhibited significantly ($P <0.05$) by all extracts of *B. napus* (Table 3.5). There were also significant differences between the various extracts ($P < 0.05$). The fresh shoot weight of each weed species decreased with increasing concentrations of water extract. Undiluted flower, stem, leaf and root extracts reduced the fresh shoot weight of *P. minor* by 58%, 100%, 61% and 43.6%, respectively and the same treatments decreased the shoot weight of C*. arvensis* by 45.3%, 82.5%, 73.5% and 44.9%, respectively and the shoot weights of *S. halepense* by 57.3%, 96.5%, 77.9% and 65.2%, respectively.

3.3.1.5. Root fresh weight (mg)

Fresh root weight was significantly decreased ($P < 0.001$) by all water extract concentrations over the control (Table 3.6). The impact of treatments on fresh root weight of weed species significantly differed. Water extract concentrations of 75% and 100% significantly reduced the fresh root weight of weed species. Undiluted flower, stem, leaf and root extracts completed inhibited the *S. halepense* fresh root 100 % inhibition. Likewise, the flower and stem extracts reduced the fresh root weight of C*. arvensis* and *P. minor* by 100%*.*

3.3.2. Experiment two

3.3.2.1. Seed germination %

The results of germination of all three weed species are shown in Fig 3.1 In most cases the seed germination percent of all three weed species were varied because of different concentration. The inhibitory effect was significantly increased ($P < 0.001$) for all three weed species at high concentration of water extract from all parts of *B. napus*. Water extract concentrations at 12.5% and 25% for all plant parts were more inhibitory to weed species than concentrations 6.25% and 3.125%. Undiluted flower extracts were the most effective against weed species compared with extracts derived from other tissues of *B. napus*. Undiluted extracts of flowers inhibited the germination at 75% and 100% concentration of *P. minor* by 60%, 43.6% , *C. arvensis* by 53.6%, 47.6% and *S. halepense* by 51.3%, 39.5% respectively.

3.3.2.2. Shoot length (cm)

Water extracts from all plant parts significantly inhibited ($P < 0.001$) shoot length of weed species Fig 3.2. Undiluted extracts of flowers, stems, leafs and roots reduced the shoot length of *P. minor*, *C. arvensis* and *S. halepense* significantly. The water

extract from flowers and stems appeared the most effective in inhibiting shoot length of *P. minor*, *C. arvensis* and *S. halepense* and it was followed by that of leaf water extracts and then roots water extracts.

All *B. napus* water extract concentrations significantly affected ($P < 0.001$) *P. minor*, *C. arvensis* and *S. halepense* shoot length Fig 3.2. Higher water extract concentrations (all tissue types) 25% and 12.5% showed the greatest suppression of shoot length of *P. minor*, C. arvensis and S. halepense significantly. The lowest suppression was recorded with the 3.125 % concentration.

3.3.2.3. Root length (cm)

Data regarding root length of *P. minor*, *C. arvensis* and *S. halepense* seedlings Fig 3.3 shown that *B. napus* water extracts from all plant parts and at different concentrations significantly reduced root length ($P < 0.001$). The highest reduction in root length of *P. minor*, *C. arvensis* and *S. halepense* seedlings is recorded with applying flowers water extracts at 12.5% and 25% concentration.

Flower extracts (25%) concentration inhibited the root length of *P. minor* by up to 70%, *C. arvensis* by up to 51.9% and *S. halepense* by up to 67.6%. Leaf extract at 25% concentration was the next best water extracts in suppressing root length of *P. minor*, *C. arvensis* and *S. halepense* seedlings and root length by up to 48%, 41.3 and 65% respectively. The lowest inhibition was documented with a concentration 3.125 % by using stems extract.

3.3.2.4. Shoot fresh weight (mg)

The data showed that *Brassica napus* extract concentration significantly ($P < 0.001$) affected shoot fresh weight of *P. minor*, *C. arvensis* and *S. halepense* seedlings Fig 3.4. All plant parts water extract significantly reduced shoot fresh weight of weed

species Fig 3.4. The highest reduction in shoot fresh weight of weed species was noted by using flowers, stems and leaves compared with using roots extracts.

The shoot fresh weight of *P. minor* and *S. halepense* was suppression up to 39.3%, 64.5% by applying flower water extract at 25% and up to 38.5%, 65.4% by applying leaf water extract at 25%.

3.3.2.5. Root fresh weight (mg)

All plant parts water extracts significantly ($p < 0.001$) decreased root fresh weight of *P. minor*, *C. arvensis* and *S. halepense* seedlings (Fig 3.5). Also, root fresh weight was significantly decreased ($p < 0.001$) by all water extract concentrations over the control (Fig 3.5).

The impact of treatments on root fresh weight of weed species significantly differed between concentrations and plant parts. Water extract concentrations of 12.5% and 25% significantly decreased the root fresh weight of weed species. Undiluted flower water extract at a concentration of 25% reduced root fresh weight of *P. minor* by 60.4%, stem water extract at a concentration 25% decreased the root fresh weight of *C. arvensis* and *S. halepense* by 94.7% and 56.4% respectively.

3.4. Discussion

In this study germination and seedling growth of *Phalaris minor* (Retz.), *Convolvulus arvensis* (L.) and *Sorghum halepense* (L.) was significantly suppressed by all water extracts made from different *B. napus* tissues (flower, stem, leaf and root). Most treatments were suppressive to seed germination of weed species. However, the highest concentrations at 75 and 100% of water extracts from flower and stem were the most effective on the seed germination in Petri dishes.

These results agree with those of Al-Sherif *et al*., (2013) who found that seed germination of *Phalaris paradoxa* was decreased with the lowest concentration of the different extracts from black mustard. However, the aqueous extract at 4% completely reduced the germination. Moreover,Tawaha and Turk (2003) document that black mustard (*Brassica nigra* L.) water extracts from different parts (leaves, stem, flower and root) decreased wild oat(*Avena fatua* L.) seed germination and seedling growth compared with the control. The results showed that seed germination and root length were affected by water extracts of brassca species. The suppressive effect on seed germination was increased with increasing concentration of water extracts from the fresh plant parts.

Futhermore, Naseem *et al.* (2009) report that isothiocyanates are most important chemical compounds in Brassicascase members to suppress the germination of many weeds species [sowthistle (*Sonchus asper* L.), scentless mayweed (*Matricaria inodora* L.), smooth pigweed (*Amaranthus hybridus* L.), barnyard grass (*Echinochloa crusgalli* L. Beauv.) and blackgrass (*Alopecurus myosuroides* Huds.)].

Also, Mason-Sedun *et al*. (1986), found that water extracts of *Brassica* tissues were mostly toxic to wheat germination. These results may due to hydrolysis of glucosinolates in *Brassica* tissues, which release numerous chemical compounds (mostly isothiocyanate), that possibly will suppress the seed germination (Brown & Morra, 1996; Yasumoto *et al*., 2010; Walsh *et al*., 2014). Fathermore Baleroni *et al*., (2000) observed that glucosinolate compounds from Brassicaceae members were capable of significantly inhibiting seed germination. They found that seed germination of alfalfa (*Medicago sativa* L.), radish and turnip (*Brassica rapa* var. rapa L.) were decreased significantly by seed water extracts of *Brassica juncea* (L.) Czern.

Bell and Muller (1973) reported that the germination of *Bromus rigidus* (Roth) seeds was significantly inhibited by allyl isothiocyante released from *B. nigra* leaves. Furthermore, the allelochemicals such as isotiocyanates have ability to inhibit the growth and development of weeds (Bangarwa and Norsworthy, 2014), also the concentration of this allelochemical is varied in different plant parts (Fahey *et al*., 2001).

Allelopathic inhibitory effects of the *Brassica napus* extracts from various parts used in this study are well reported in the literature (Kim *et al*. 1993; Batish *et al*. 2002; Mughal, 2000; El-Beltagi and Mohamed, 2010; Embaby *et al*., 2010). The water extracts from different plant parts by concentration 25% in the experiment two mostly more effective on seedling growth of weed species compared with the same concentration in experiment one.

This differences in the results of two experiments may due to several factors affecting the amount of allelochmicals in *B. napus* tissues such as; light, temperature and timing of crop sowing or maybe also that the field crop was treated with agrochemicals. For instance, Justen and Fritz (2013) reported that the glucosinolate levels have been increased by increasing the temperatures.

3.5. Conclusion

In this study the using different concentration of water extracts from different parts of *B. napus* revealed that all concentrations showed significant inhibition of germination, shoot and root length and shoot and root weight for all weed species compared to the control. The greatest effect was observed with using hiegh concentration from all parts of *B. napus* especially with using the flower and stem extract at 100% and 75%.

Stem extracts at 75% and 100% concentrations completely inhibited the root length of *P. minor* and *S. halepense*. *P. minor* weight was the most effecitev weed by great concentration from all plant parts. Flower, stem, leaf and root extracts completed inhibited the *S. halepense* fresh root by 100 % inhibition. Also, the flower and stem extracts reduced the fresh root weight of C*. arvensis* and *P. minor* by 100%.

Table 3.2. Effect *Brassica napus* (L.) water extracts of various concentrations on the seed germination % over the control of *Phalaris minor* (Retz.) (P.m.), *Convolvulus arvensis* (L.) (C.a.) and *Sorghum halepense* (L.) (S.h.). n = 25 for each treatment**.**

Treatments	Percentage of Seed germination inhibition over the control											
	Flower extracts			Stem extracts			Leaf extracts			Root extracts		
	P.m.	C.a.	S.h.	P.m.	C.a.	S.h.	P.m.	C.a.	S.h.	P.m.	C.a.	S.h.
25%	74.75	45.3	72.5	60.3	29.1	53.9	22.9	41	61.3	24.75	14.1	52.9
50%	82.8	60.5	73.8	93.8	63.9	91.1	62.3	61.2	76	47.3	26.6	67.1
75%	87	73.3	79.75	99.4	81.5	94	66.7	69.2	90	69.75	42.9	79.9
100%	91.4	83	84.9	100	95.05	99.4	78.7	75.75	95.6	80.75	56.5	86.6
SEM	3.905	3.917	3.797	4.691	5.443	4.771	5.568	5.459	4.708	5.037	3.519	3.615
C.V.%	75.10	52.37	70.31	125.6	72.54	122.7	55.72	61.92	94.16	54.48	29.45	66.22
P value	< 0.001	< 0.001	0.002	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001

Table 3.3. Effect *Brassica napus* (L.) water extracts of various concentrations on the shoot length (cm) over the control of *Phalaris minor* (Retz.) (P.m.), *Convolvulus arvensis* (L.) (C.a.) and *Sorghum halepense* (L.) (S.h.). n = 25 for each treatment**.**

Treatments	Percentage of shoot length inhibition over the control											
	Flower extracts			Stem extracts			Leaf extracts			Root extracts		
	P.m.	C.a.	S.h.	P.m.	C.a.	S.h.	P.m.	C.a.	S.h.	P.m.	C.a.	S.h.
25%	25.5	14	43.8	11.5	20.7	35.1	12.5	21.1	11.01	18.2	18.3	23
50%	30	29.5	42.9	48.9	32	74.9	31.9	36.3	32.1	35	28.6	38.4
75%	34.5	32.3	54	95.2	50	84.7	48.8	67.15	51.55	51.5	33.9	53
100%	58	45.3	57.3	100	82.5	96.5	61.6	73.5	77.9	43.6	44.9	65.2
SEM	0.266	0.470	0.507	0.657	0.651	0.989	0.329	0.713	0.701	0.241	0.355	0.578
C.V.%	30.03	26.02	37.41	95.13	36.92	105.9	37.38	50.14	39.75	24.28	20.93	40.95
P value	< 0.001	0.003	0.010	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001

Table 3.4. Effect *Brassica napus* (L.) water extracts of various concentrations on the root length (cm) over the control of *Phalaris minor* (Retz.) (P.m.), *Convolvulus arvensis* (L.) (C.a.) and *Sorghum halepense* (L.) (S.h.). n = 25 for each treatment**.**

Treatments	Percentage of root length inhibition over the control											
	Flower extracts			Stem extracts			Leaf extracts			Root extracts		
	P.m.	C.a.	S.h.	P.m.	C.a.	S.h.	P.m.	C.a.	S.h.	P.m.	C.a.	S.h.
25%	58.4	54.75	79.7	26.1	44.35	81.3	32	50.1	27.6	21.75	45.1	58.9
50%	74	84.6	95.8	81.1	71.7	98.3	51.4	64.1	64	38.2	56	78.1
75%	88.5	93	100	100	84.6	100	62.8	70.9	90	45.6	56.4	95
100%	94	98.3	100	100	100	100	76.8	85	100	56.9	72.6	100
SEM	0.293	0.859	0.352	0.438	0.864	0.231	0.309	0.663	0.336	0.190	0.556	0.351
C.V.%	107.9	115.2	164.6	119.9	90.97	182.0	52.9	67.28	90.17	30	52.14	114.8
P value	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001

Table 3.5. Effect *Brassica napus* (L.) water extracts of various concentrations on the fresh shoot weight (mg) over the control of *Phalaris minor* (Retz.) (P.m.), *Convolvulus arvensis* (L.) (C.a.) and *Sorghum halepense* (L.) (S.h.). n = 25 for each treatment**.**

Treatments	Percentage of fresh shoot weight inhibition over the control											
	Flower extracts			Stem extracts			Leaf extracts			Root extracts		
	P.m.	C.a.	S.h.	P.m.	C.a.	S.h.	P.m.	C.a.	S.h.	P.m.	C.a.	S.h.
25%	36.2	35.1	62	12.8	28.4	22.7	17	18	27.8	13.5	24.6	21.8
50%	44	43.6	68.7	55.8	47.8	78.9	31.6	34.9	67	27.4	35.6	43.2
75%	48.4	46.3	72.1	96.9	53.7	77.5	41.4	49.7	75.2	34.7	45.4	69.7
100%	65.2	60.4	71.4	100	76.2	94.7	60	63.9	88.5	45.5	55.5	77.2
SEM	0.463	6.08	2.46	0.849	6.53	1.52	0.412	4.48	2.74	0.262	4.7	1.38
C.V.%	36.39	35.81	75.73	96.34	47.34	108.4	33.49	32.08	72.53	19.55	30.13	52.22
P value	< 0.001	0.003	0.031	< 0.001	0.029	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001

Table 3.6. Effect *Brassica napus* (L.) water extracts of various concentrations on the fresh root weight (mg) over the control of *Phalaris minor* (Retz.) (P.m.), *Convolvulus arvensis* (L.) (C.a.) and *Sorghum halepense* (L.) (S.h.). n = 25 for each treatment**.**

Treatments	Percentage of fresh root weight inhibition over the control											
	Flower extracts			Stem extracts			Leaf extracts			Root extracts		
	P.m.	C.a.	S.h.	P.m.	C.a.	S.h.	P.m.	C.a.	S.h.	P.m.	C.a.	S.h.
25%	69.8	41.7	62	43.2	62.3	84.4	34.4	48	25	22.5	50.2	56.1
50%	78.9	63.9	94.7	91.9	68.4	99.85	49.5	61.1	70.4	50	55.9	73.3
75%	96	83.4	100	100	84.5	100	70.7	76.3	94.6	59.1	67.7	98.1
100%	97	96.3	100	100	100	100	83.5	82.8	100	79.8	70.5	100
SEM	0.463	6.08	2.46	0.849	6.53	1.52	0.412	4.48	2.74	0.262	4.7	1.38
C.V.%	36.39	35.81	75.73	96.34	47.34	108.4	33.49	32.08	72.53	19.55	30.13	52.22
P value	< 0.001	0.003	0.031	< 0.001	0.029	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001

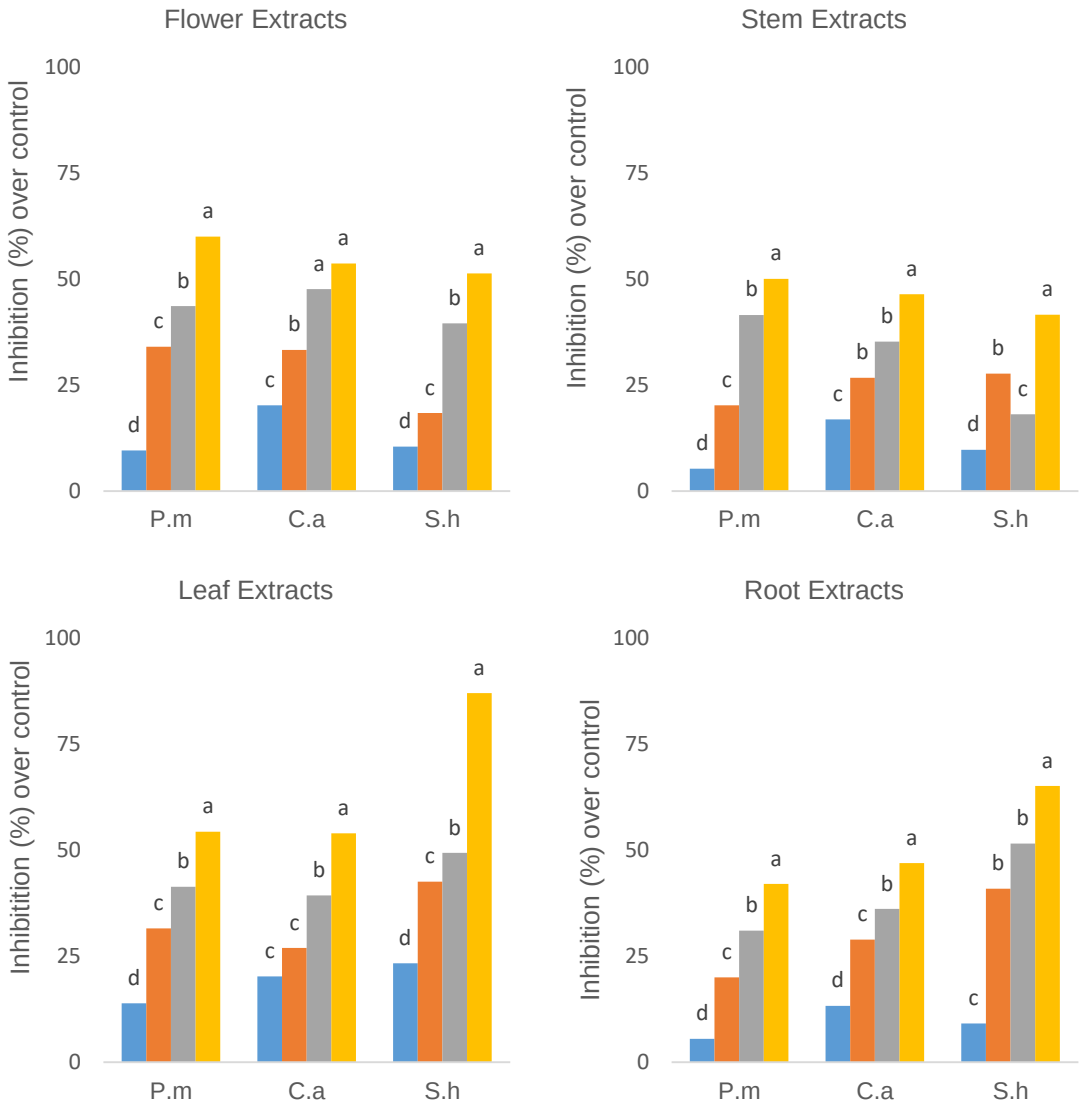

Figure 3. 1. Effect *Brassica napus* (L.) water extract of various concentrations on the seed germination (%) over the control of *Phalaris minor* (P.m.), *Convolvulus arvensis* (C.a.) and *Sorghum halepense* (S.h.) 14 days after sowing. 3.125% 6.25% 12.5% 25%. Bars with the same letter are not significantly different according to Duncan's multiple range test ($P < 0.05$). n = 25 for each treatment.

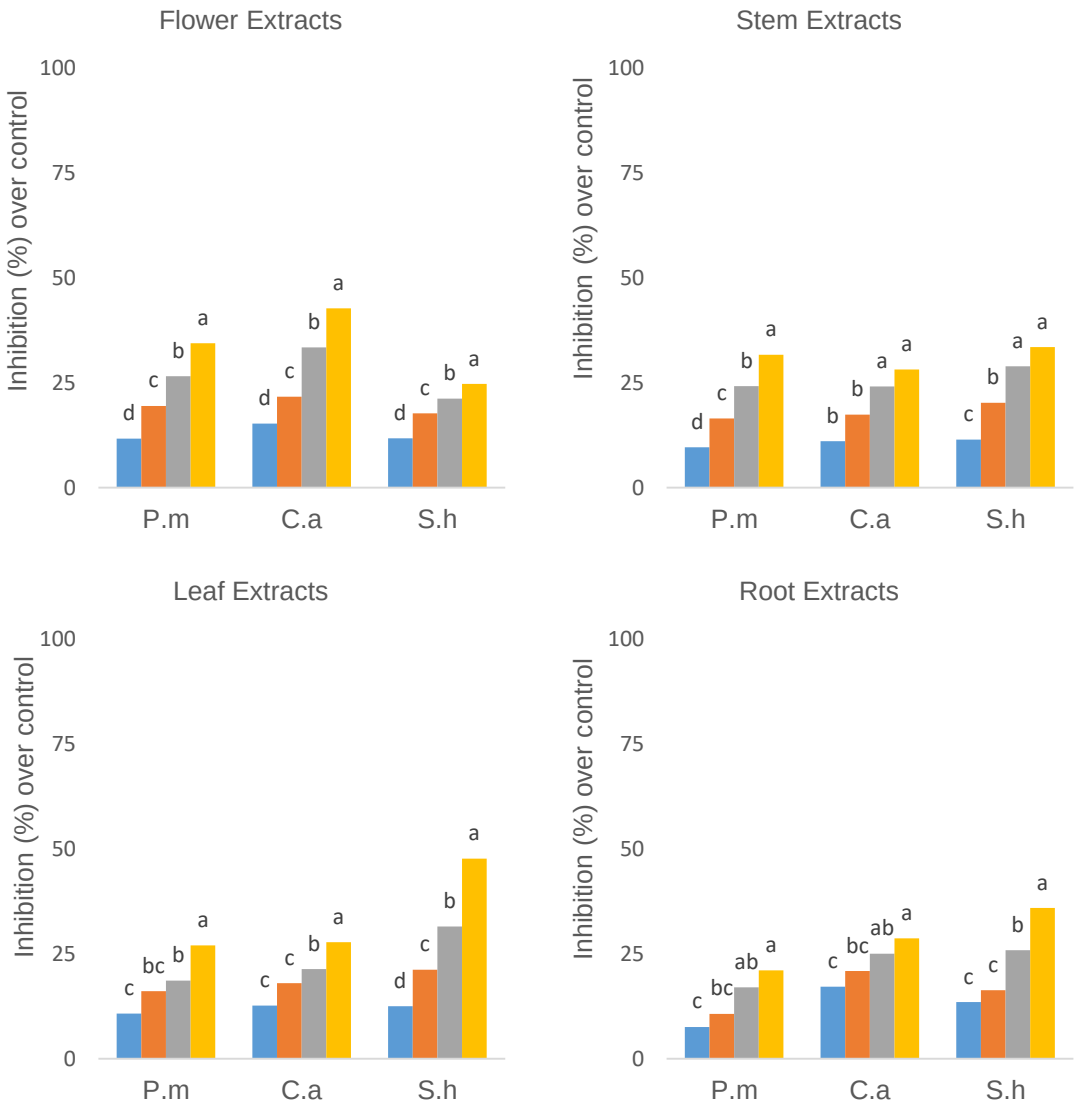

Figure 3. 2. Effect Brassica napus (L.) water extract of various concentrations on the shoot length (cm) over the control of Phalaris minor (P.m.), Convolvulus arvensis (C.a.) and Sorghum halepense (S.h.) 14 days after sowing. 3.125% 6.25% 12.5% 25%. Bars with the same letter are not significantly different according to Duncan's multiple range test ($P < 0.05$). n = 25 for each treatment.

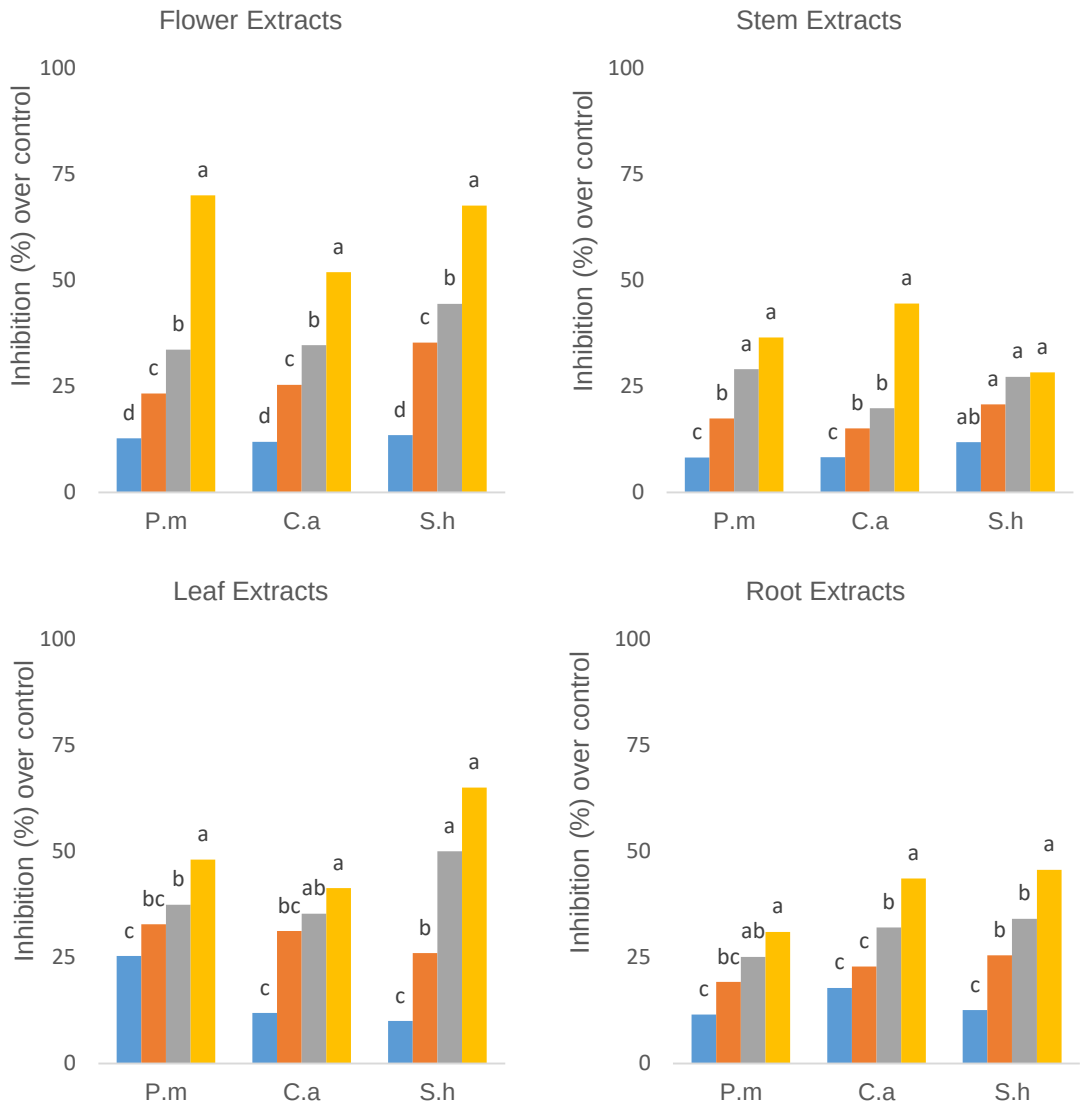

Figure 3. 3. Effect *Brassica napus* (L.) water extract of various concentrations on the root length (cm) over the control of *Phalaris minor* (P.m.), *Convolvulus arvensis* (C.a.) and *Sorghum halepense* (S.h.) 14 days after sowing. 3.125% 6.25% 12.5% 25%. Bars with the same letter are not significantly different according to Duncan's multiple range test ($P < 0.05$). n = 25 for each treatment.

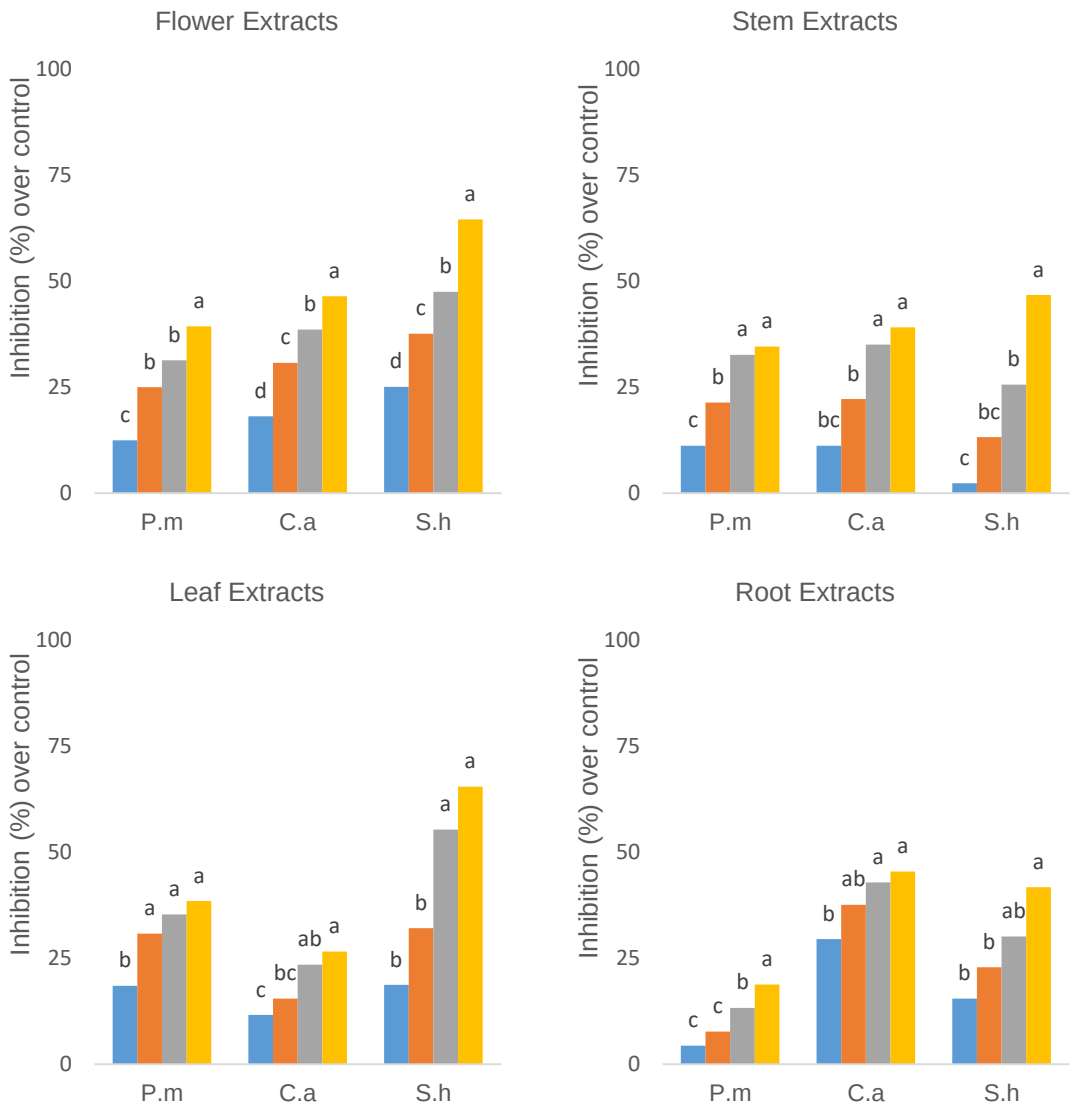

Figure 3. 4. Effect *Brassica napus* (L.) water extract of various concentrations on the shoot fresh weight (mg) over the control of *Phalaris minor* (P.m.), *Convolvulus arvensis* (C.a.) and *Sorghum halepense* (S.h.) 14 days after sowing. 3.125% 6.25% 12.5% 25%. Bars with the same letter are not significantly different according to Duncan's multiple range test ($P < 0.05$). n = 25 for each treatment.

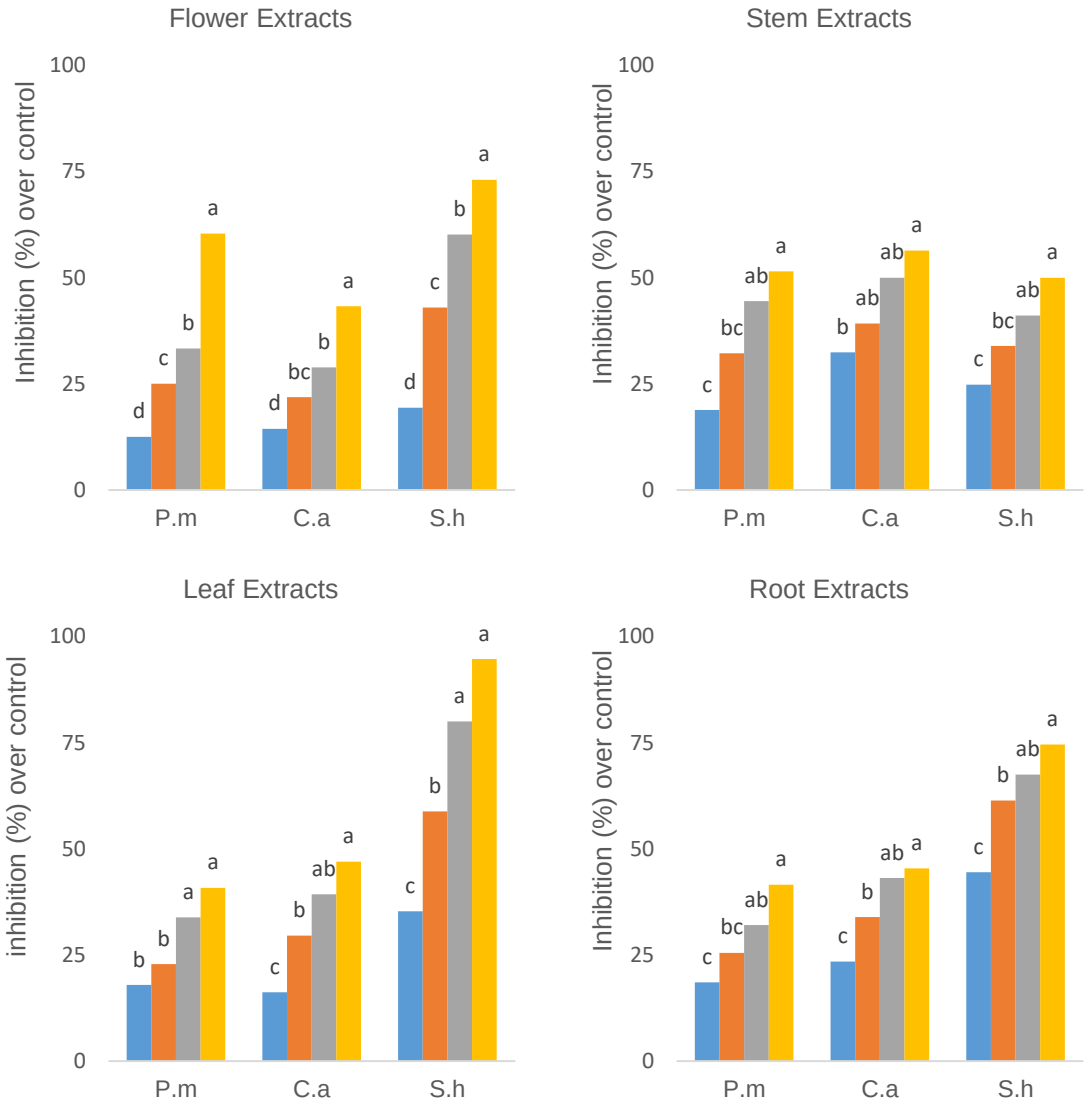

Figure 3. 5. Effect *Brassica napus* (L.) water extract of various concentrations on the root fresh weight (mg) over the control of *Phalaris minor* (P.m.), *Convolvulus arvensis* (C.a.) and *Sorghum halepense* (S.h.) 14 days after sowing. 3.125% 6.25% 12.5% 25%. Bars with the same letter are not significantly different according to Duncan's multiple range test ($P < 0.05$). n = 25 for each treatment.

Chapter 4

4. Allelopathic effect of *Brassica napus* L. tissues collected at different development stages on the suppression of weed species

4.1. Introduction

As revealed in Chapters 2 and 3, it is clear that *Brassica napus* had a significant inhibitory effect on all three weed species when treated by water extracts from different plant parts at a range of concentration. In order to provide a deeper understanding of the mechanism of this inhibitory effect on weed species inhibition further studies were conducted on water extracts from different *B. napus* tissues (leaves, stems, flowers and roots) which were collected during different growth development time.

It is well documented that members of the Brassicaceae family possess allelopathic metabolites whose profile and quantity is affected by the growth stages of the plant and the type of tissue from which they are extracted from (Chapter 1).

The production of chemical substance (allelochemicals) depends on the development stages of plants and several environmental conditions (Tang *et al*., 1995). Previously, Jafarieyazdi and Javidfar (2011) used water extracts from three species of brassica (*B. napus*, *B. rapa* and *B. juncea*) collected at two development stages significantly reduced sunflower germination, germination rate, shoot and root length and their weight. The most sensitive parameter to these water extracts was the root length.

Several researchers have shown that the profile of glucosinolates and their concentration in species within the Brassicaceae were affected by plant parts and growth development stages (Booth and Walker 1992; Ludwig-Müller *et al*., 1999; Bartlet *et al*., 1999; Hasegawa *et al*., 2000; Bellostas *et al*. 2007; Alnsour *et al*., 2013; Park *et al*., 2013). Rosa *et al.* (1997) observed the glucosinolate content to be about 1% of dry weight in some parts of the *Brassica spp* and 10% in the seed.

Glucosinolate concentration in the Brassica species plant were significantly higher in the young leaves, shoots and silique walls, possibly due to the biosynthetic activities which are higher at these stages (Bennett *et al*., 1995; Bellostas *et al*., 2004). Similar observations have been made by Booth *et al* (1991) who showed that glucosinolate levels decreased in the mature leaves, especially at the flowering and seeds stage.

On other hand, the activity of plant myrosinase and its isoenzymes may demonstrate significant variances between and within Brassica species and cultivars, in different parts of the plants and between seasons. (Charron *et al*., 2005). Myrosinase activity was the highest in leaves, followed by developing seeds compared with the mature seeds (Atwal *et al*., 2009). During early seedling growth the enzyme activity was significantly increased in *Brassica napus* tissues (James and Rossiter, 1991).

4.2. Aims

i. To quantify the effect of *Brassica napus* water extracts from different parts (stem, leaf, root and flower) collected at different growth development times on weed species suppression.

ii. Determine the glucosinolate profile and myrosinase activity from different parts of *Brassica napus* at different growth development times to develop better weed management strategies for using *B. napus.*

4.3. Materials and methods

4.3.1. Plant material

Oilseed rape (*Brassica napus L.*) cv. PR46W21 were planted in 100 pots (19 cm-diameter) March 2013. Pots were filled using John Innes No. 2 sterilised loam based compost (Norwich, UK). Five seeds were planted at 01/02/2014 in each pot and placed in a poly-tunnel at Harper Adams University Edgmond, Newport, Shropshire, England, UK. Plants were watered when required. After two weeks, the plants were thinned to 2 plants per pot.

Plants were collected at eight different growth times:

1- Time 1 (T1) at 01/03/2014.
2- Time 2 (T2) at 15/03/2014.
3- Time 3 (T3) at 01/04/2014.
4- Time 4 (T4) at 15/04/2014.
5- Time 5 (T5) at 01/05/2014.
6- Time 6 (T6) at 15/05/2014.
7- Time 7 (T7) at 01/06/2014.
8- Time 8 (T8) at 15/06/2014.

4.3.2. Plant sampling and processing

Fresh *Brassica napus* leaves, stems and roots from T1 to T8 and flowers from T7 and T8, collected and transported to the laboratory using dry ice to inactivate endogenous myrosinase enzyme, and then and placed into separate plastic bags and stored at -80 °C before freeze-drying. Frozen samples were dried using a GVD6/13 MKI freeze dryer, (GIROVAC Ltd, North Walsham, UK) for 6-7 days before being milled to a fine powder in a micro-grinder (Retsch GmbH Cyclone Mill-Twister,

Haan, Germany). Each milled samples was placed in a separate plastic bag and stored below -18° until required for water extracts preparation and glucosinolates analysis.

4.3.3. Preparation of water extracts

Water extracts were prepared based on the method described by AL- Sharif *et al*., (2013) with some minor modifications. One gram of freeze-dried material from the leaves, stems, roots and flowers from each plant development stages were soaked seperately in 100 ml distilled water for 24 h at room temperature (20^{o}C± 2) to obtain water extracts. This solution was filtered through two sheets of filter paper (Whatman No.2) to remove the solid organic material.

4.3.4. Bioassay

Seeds (250) of test weeds [*Phalaris minor* (Retz.) (canary grass), *Convolvulus arvensis* (L.) (field bindweed) and *Sorghum halepanses* (L.) (Johnsongrass)] were prepared as previously described (Chapter 2, section 2.2.2).

Ten ml of extract solution from each plant parts collected at different development stages was added to each petri dish and distilled water was used as the control. All Petri dishes were placed randomly in plant growth chambers (Sanyo MLR) at 25°C in dark conditions. Treatments were arranged in a completely randomized design (CRD) with factorial arrangements in six replications (5 Plant water extract types x 8 times from (stem, leaf and root) and 2 times from flower for each weed species separetely.

4.3.5. Assessment

Germination, shoot and root length and fresh weight of seedlings were measured as previously described (Chapter 2, section 2.2.3).

4.3.6. Determination of glucosinolate

High Performance Liquid Chromatography (HPLC) (Agilent HPLC series 1100, Plate 4.1) was used to determine the different glucosinolates in *Brassica napus*. The following reagents were used for extraction and analysis of glucosinolates and obtained from Sigma Aldrich®, UK.

- Sinigrin monohydrate
- 2M acetic acid
- Formic acid
- Imidazole
- Sulfatase (β-glucuronidase) Type H-1 from *Helix pomatia* (10 KU)
- Sephadex A-25
- Sephadex C-25
- Absolut Methanol
- Acetonitrile

4.3.6.1. Extraction of glucosinolates from *Brassica napus* material

Glucosinolate concentrations from freeze-dried *Brassica napus* tissues were determined by using procedure described in ISO 9167-1-1992, and Brown *et al*. (2003).

Three hundred mg of freeze-dried plant tissue were transferred to a 15 ml polypropylene tube and heated in a water bath at 75°C for 1 min. Four ml of boiling

methanol 70% v/v were added to the polypropylene tubes containing freeze-dried plant tissue to deactivate the myrosinase enzyme. Tubes were incubated at 75°C for a further 10 min during which the tubes at were gently shaking at regular intervals before being allowed to cool. The tubes were centrifuged at 5000 g for 10 min at 4˚C (Beckman AvantiTM 30 High Speed Compact Centrifuge) and each sample was extracted twice and the supernatant was combined in a 15 ml polypropylene tube and 1µmol (200µl from a 5mM stock solution) of internal standard solution of sinigrin was added to the extract. The combined extracts were gently mixed and the volume adjusted to 5 ml.

4.3.6.2. Purification and desulfation

DEAE-Sephadex A-25 (Sigma Aldrich®, UK) is one of the weak anion exchangers with a diethyaminoethyl, hence the ion exchange stage was important in order to remove contaminating hydrophilic impurities that might interact with detection and quantification as well as binding to intact glucosinolates. To prepare the anion exchange resin column, a Pasteur pipette was placed on a stand and then a glass wool plug was placed in the constricted end of each pipette; 0.5 ml of DEAE-Sephadex A-25 resin suspension was added to the pipette. The column was rinsed with 2 ml of 6 M imidazole formats and allowed to derail, after which, it was washed twice with 1 ml deionised water. One ml of the extract was added to the prepared column followed by 2x1 ml aliquots of sodium acetate buffer at pH 4, which were allowed to drain after each addition. Then 75 µl of diluted purified sulfatase solution type *H-1* from *Helix pomotia* was added to each column, and the column was covered by parafilm and allowed to incubate overnight at room temperature. The desulfo-glucosinolates were eluted with 3 x 0.33 ml aliquots of deionize water. Water was allowed to drain into HPLC vials after each addition and the vials were then capped for HPLC analysis.

4.3.6.3. HPLC analysis

The different glucosinolates and their concentrations in rapeseed were determined using High Performance Liquid Chromatography (HPLC) (Agilent HPLC series 1100, Waldbronn, Germany). A reverse-phase gradient HPLC column Spherisop® RP-C18 ODS-2 (250X 4.6mm, Phenomenex Inc. Macclesfield, UK) with a particle size of 5 µm was used. The mobile phase consisted of eluent-A (deionised water) and eluent-B (acetonitrile/ deionised water, 70:30 v/v). Ten µl of each sample was auto-injected into the column. A linear gradient was carried out from 0-30% eluent B over a period of 18 min, and held at 30% eluent B for 1min prior to returning to 0% eluent B for 1min. This was followed by an equilibrium establishment over 6 min and a post – run time of 2 min. The desulfo-glucosinolates were determined at a flow rate of 1.5 ml min^{-1} at 30 ^{o}C and a *UV* wavelength of 229 nm. Glucosinolate concentrations were calculated using equation (4.2) where, Ag= peak area of relative GSL, As = peak area of the internal standard hused, n= amount (µmol) of the IS used, m= Mass (g) of freeze-dried test sample and RRF = relative response factor of the glucosinolate. Total and individual glucosinolates are expressed as µmol g^{-1}.

$$\text{GSL concentrations} = \frac{\text{Ag}}{\text{As}} \times \frac{\text{n}}{\text{m}}\ \text{RRF} \qquad (4.2)$$

4.3.7. Determination of myrosinase activity

The myrosinase activity was based on the release of glucose through the reaction between sinigrin and myrosinase. Myrosinase activity was determined using a spectrophotometer in water extracts prepared from different parts of *B. napus* (leaves, stems, roots and flowers), according to enzymatic assay procedure previously described with minor modifications (Sigma Aldrich®, UK). Plant tissue

samples were prepared as described previously (see section 4.3.2) and were used to determine the myrosinase activity.

Sample solutions were prepared by adding 0.2 mg from grounded leaf, stem, root and flower of *B. napus* to 5 ml deionized water and centrifuged for 5 min at 5000 g for 10 min at 4˚C (Beckman AvantiTM 30 High Speed Compact Centrifuge) to remove debris. The supernatant was moved in a 10 ml polypropylene tube and stored at 4˚C in the ice box until used. Sinigrin 4.8 mM solution was prepared by dissolving a 199.39 mg of potassium allylglucosinolate monohydrate in 100 ml of 126 mM sodium phosphate buffer with 37 mM citric acid and 1 mM ascorbic acid adjusted to pH 6 at 25°C. Nine ml from of sinigrin solution was added to 15 ml polypropylene tube and equilibrated to 25 ˚C and 1 ml of the supernatant was added to the sinigrin solution, immediately mixed by inversion and incubated for exactly 10 min. Three mg of glucose was dissolved in 3 ml of deionized water and added into suitable cuvettes equilibrated to 25 ˚C than 0.10 ml from mixed sinigrin solution with supernatant was add to glucose solution in cuvettes and immediately mixed and record the increase in A_{340nm} for approximately 5-10 min until constant. Myrosinase activity was calculated using equation (4.3) where,

10 = Total volume (in milliliters) of mixed solution (9 ml sinigrin + 1ml supernatant)

3.1 = Total volume (in milliliters) of mixed solution (3 ml glucose solution + 0.10 ml of mixed solution of sinigrin and supernatant).

df = Dillution factor

1 = volume of supernatant solution (in milliliters)

0.1 = volume of mixed sinigrin and supernatant solution (in milliliters)

10 = Time of incubated of mixed sinigrin and supernatant solution (in minutes)

$$\text{Units/ml enzyme} = \frac{(\Delta \text{A340nm Test})\ (10)\ (3.1)\ (\text{df})}{(1)\ (0.1)\ (10)} \qquad (4.3)$$

4.3.8. Statistical analysis

Treatments were arranged in a completely randomized design (CRD) with factorial arrangements in six replications [5 plant water extract types x 8 plant development stages from 1-8 for (stem, root and leaf)] and from 7-8 for flower. A general analysis of variance (ANOVA) two –way was carried out to analyse the experiment results from each weed sepcies separately using GenStat® 15th Edition (VSN international, Hemel Hemstead, UK). Also, two –way ANOVA was carried out to analyse the results of Glucosinolatet concetrations and myrosinase activity using GenStat® 15th Edition (VSN international, Hemel Hemstead, UK) [4 plant parts x 8 plant development stages from 1-8 for (stem, root and leaf)] and from 7-8 for flower with six replications. Where necessary, data were log10-transformed to normalise residuals. A Duncan multiple range test was used to compare the differences between means of treatments at level ($P < 0.05$). Regression analysis was also applied to determine the relationship between different characters

4.4. Results

4.4.1. Bioassay

4.4.1.1. Seed germination %

The effect of using water extracts from different parts of *Brassica napus* at different development stages on seed germination% of *P. minor* (Retz.), *C. arvensis* (L.) and *S. halepense* (L.) is demonstrated in figures 4.1, 4.2 and 4.3. The results of the data analysis showed that water extracts from all parts of *Brassica napus*, collected at different development stages, significantly ($p<0.001$) inhibited seed germination % of all three weed species as compared with control (Figure 4.1, 4.2 and 4.3) and (Table 4.1). Seed germination of *P. minor* was completely inhibited in petri dishes

when treated with water extract from flowers at T7 and T8 and germination was reduced by 100%. Also, water extract from stems was found to be more effective on *P. minor* germination at theT1, T3 and T6 development stages when compared with water extract from leaves and roots at the same development stages, with germination suppressed by up to 82%, 88.6% and 84.5% respectively.

Almost a similar trend was found with applying water extracts from all plant parts in T7 and T8 on *C. arvensis* and *S. halepense* with the water extract from flowers appearing to be the most effective in suppressing the germination (Figure 4.2 and 4.3). The germination of *C. arvensis* was inhabited by up to 97.9% and 100% respectively, while also *S. halepense* germination was reduced by up to 96.9% and 98.9% respectively.

The extract from stems at T1 was the most effective in suppressing the germination of *C. arvensis* compared with leaf and root extracts. However, in T2 and T3, the greatest inhibition of *C. arvensis* germination was demonstrated when leaf and root extracts were applied. Also, the water extract from stems and leaves in T7 and T8 revealed a strong effect on *C. arvensis* germination compared with other development stages. However, no significant effect was found between stem, leaf and root extracts in T4 and T5 (Figure 4.2). On the other hand, *S. halepense* germination was highly inhibited by applying water extract from stems, leaves and roots in T1, T2, T3 and T5. In T6, stem and root extracts were the most effective on *S. halepense* germination when compared with application of leaf extracts (Figure 4.3). The relationship between and seed germination % of *P. minor C. arvensis* and *S. halepense* and total GSL concentration was weak and non-significant (R^2 = 0.0831, R^2 = 0.0136 and R^2 = 0.0081) (Figure 4.4 A, B and C)

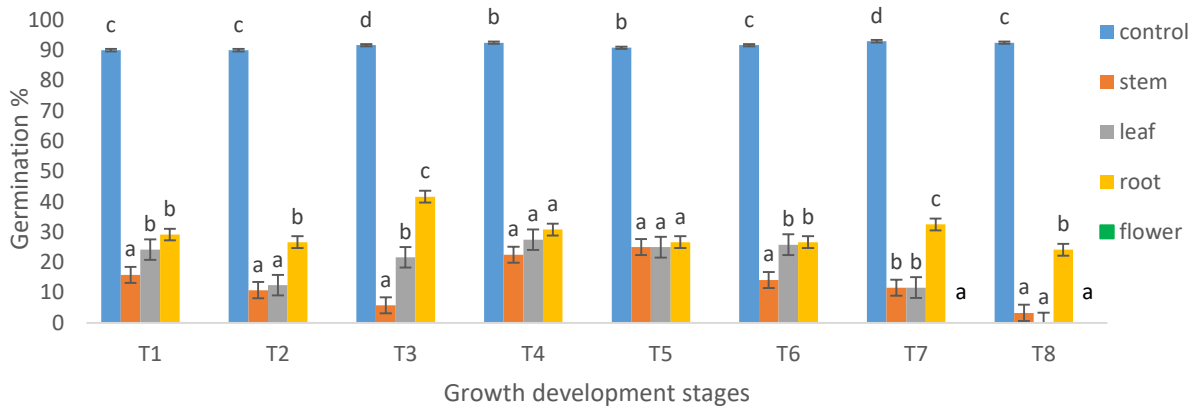

Figure 4.1. Effect of *B. napus* water extracts from (stem, root and leaf) collected at different times after germination (T1-T8) and flower collected at (T7 and T8) on the germination (%) of *Phalaris minor* 14 days after sowing. Bars with the same letter in each growth stage are not significantly different according to Duncan's multiple range test ($P < 0.05$).). Error bars represent the standard error of the mean. n = 192 for each treatment.

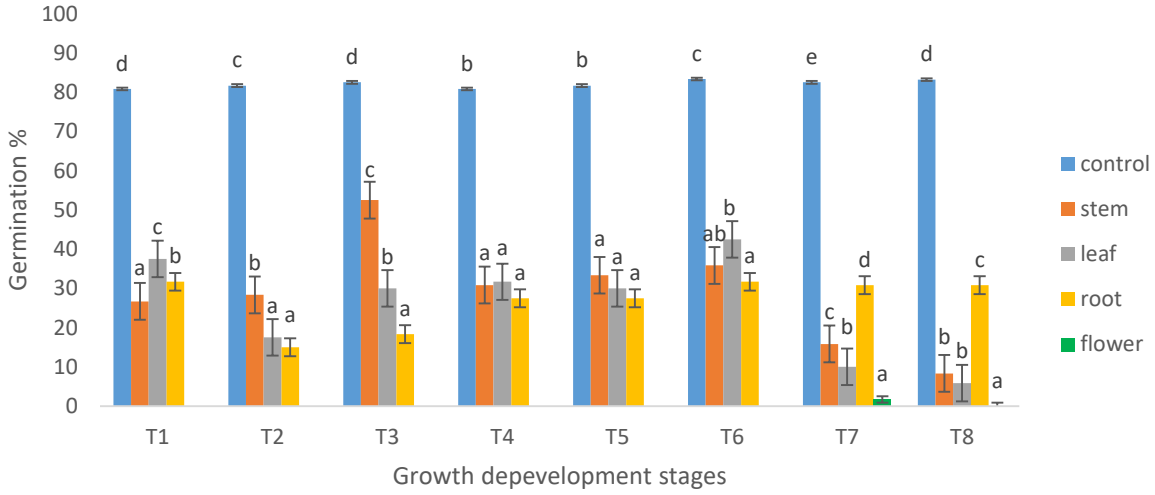

Figure 4.2. Effect of *B. napus* water extracts from (stem, root and leaf) collected at different times after germination (T1-T8) and flower collected at (T7 and T8) on the germination (%) of *Convolvulus arvensis* 14 days after sowing. Bars with the same letter in each growth stage are not significantly different according to Duncan's multiple range test ($P < 0.05$).). Error bars represent the standard error of the means. n = 192 for each treatment.

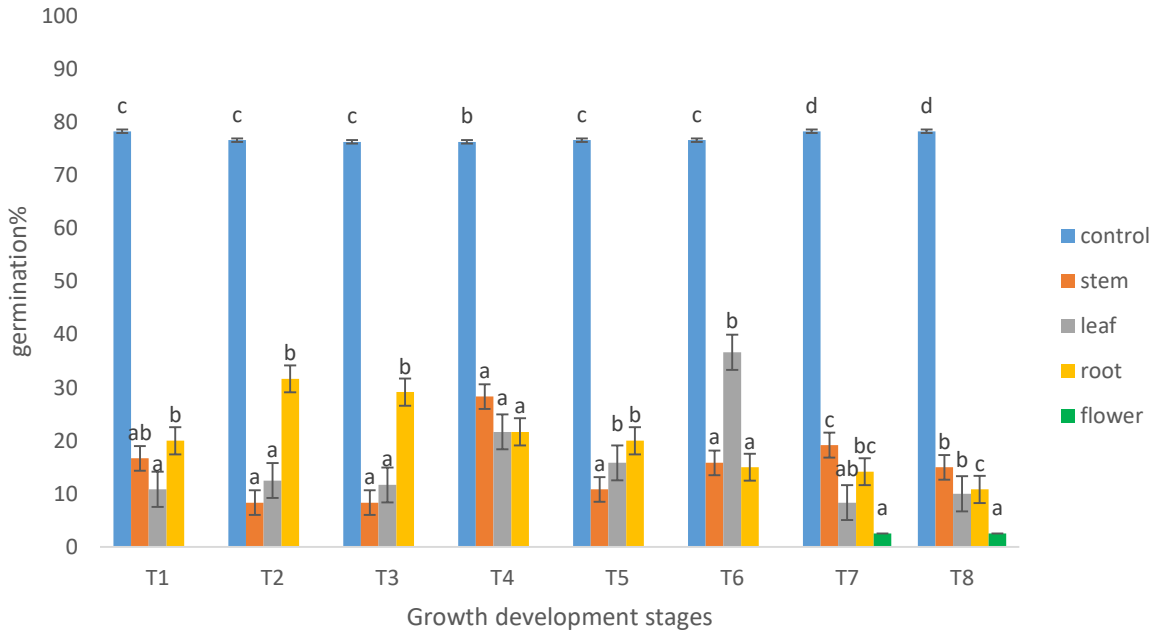

Figure 4.3. Effect of B. napus water extracts from (stem, root and leaf) collected at different times after germination (T1-T8) and flower collected at (T7 and T8) on the germination (%) of Sorghum halepense 14 days after sowing. Bars with the same letter in each growth stage are not significantly different according to Duncan's multiple range test ($P < 0.05$).). Error bars represent the standard error of the mean. n = 192 for each treatment.

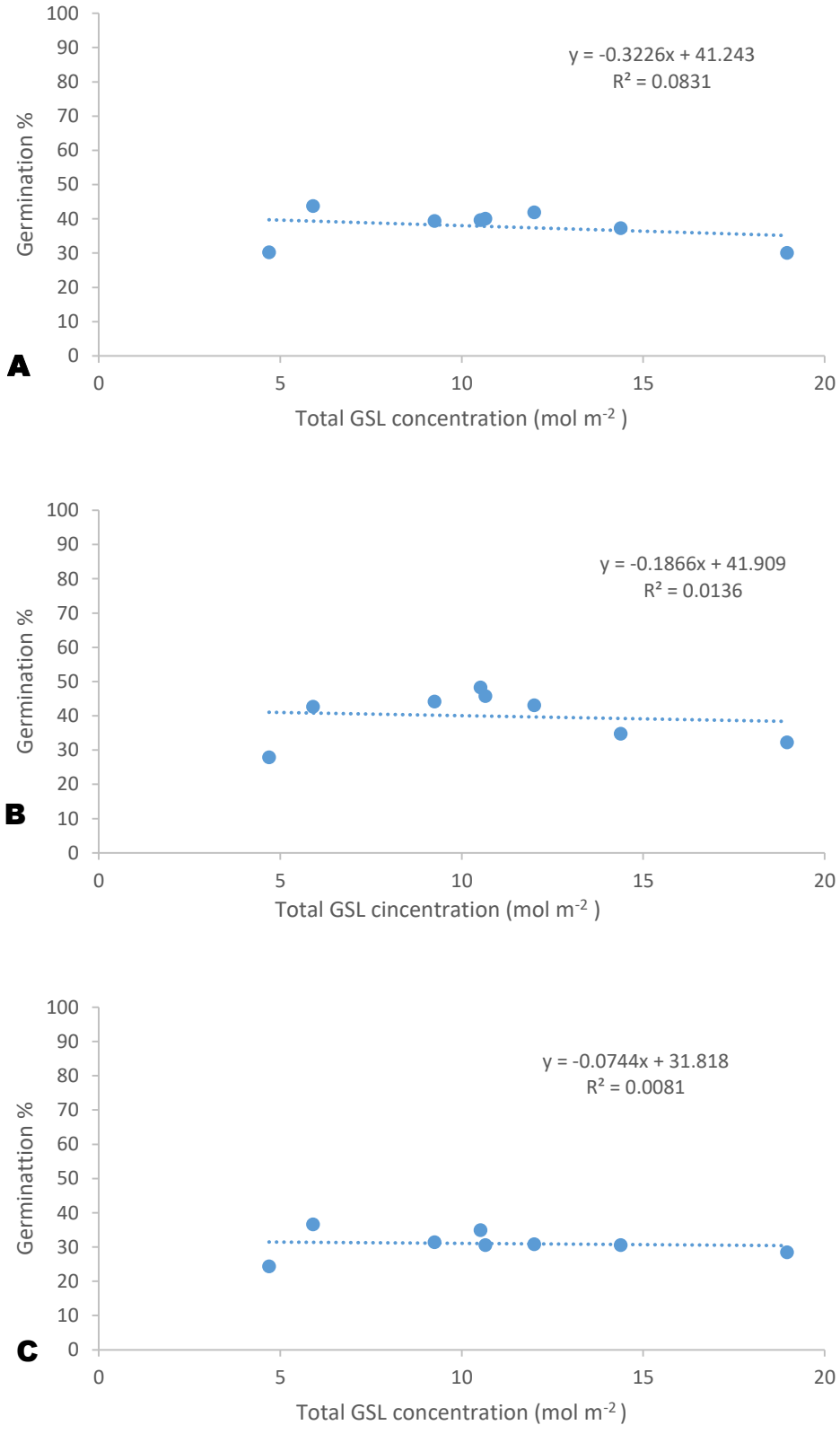

Figure 4.4. Relationships between seed germination (%) of *Phalaris minor* (A), *Convolvulus arvensis* (B), *Sorghum halepense* (C) and Total glucosinolate (GSLs) concentration during different development stages after 14 days.

4.4.1.2. Shoot length (cm)

Figures 4.5, 4.6 and 4.7 and Anova variance table 4.2 illustrate the effect of water extracts from different plant parts at different development stages. The shoot length data of all three weed species *P. minor* (Retz.), *C. arvensis* (L.) and *S. halepense* (L.) showed significant (*P< 0.001*) effects from all water extracts from all different plant parts at different development stages on shoot length as compared with the control.

It was observed that flower water extract at T7 and T8 completely inhibited the germination of *P. minor* by 100% and that means no seeds germinated under these treatments, thus the *P. minor* shoot length reduced by 100%. The next best treatment was leaf water extract at T7 and T8, and the shoot length of *P. minor* was suppressed by 53.5% and 100% respectively. Additionally, water extract from stems at T1, T2, T3 and T6 significantly affected *P. minor* shoot length and were reduced up to 36.2%, 62.5%, 29.8% and 16.4% respectively.

Meanwhile, similar results were observed with applying flower water extract at T7 and T8 on *C. arvensis* and *S. halepense*. The shoot length of *C. arvensis* was reduced up to 93.8% and 100% respectively, while reductions in *S. halepense* shoot length were 87.3% and 93.4 respectively (Figure 4.6 and 4.7). Root water extract appeared to be the most effective treatment in suppressing *C. arvensis* shoot length at T1, T2, T4, T5 and T6, followed by water stem extracts. Moreover, water extracts from stems, leaves and roots at T3, T5 and T6 significantly reduced *S. halepense* shoot length but there is no significant differences between the treatments. Stems extract at T1 and T2 was the most effective on *S*. *halepense* shoot length. A weak realationship was ound between the shoot length of all three weed species *P. minor* (Retz.), *C. arvensis* (L.) and *S. halepense* (L.) and total GSL concentration (R^2 = 015, R^2 = 0.002 and R^2 = 0.12) respectively (Figure 4.8 A, B and C)

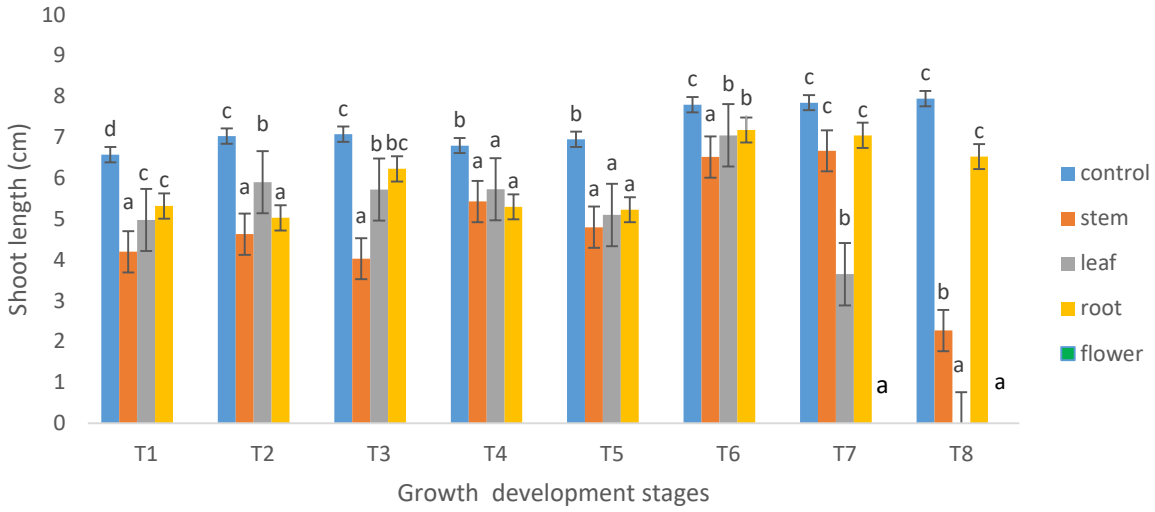

Figure 4.5. Effect of *B. napus* water extracts from (stem, root and leaf) collected at different times after germination (T1-T8) and flower collected at (T7 and T8) on the shoot length (cm) of *Phalaris minor* 14 days after sowing. Bars with the same letter in each growth stage are not significantly different according to Duncan's multiple range test ($P < 0.05$).). Error bars represent the standard error of the mean. n = 192 for each treatment.

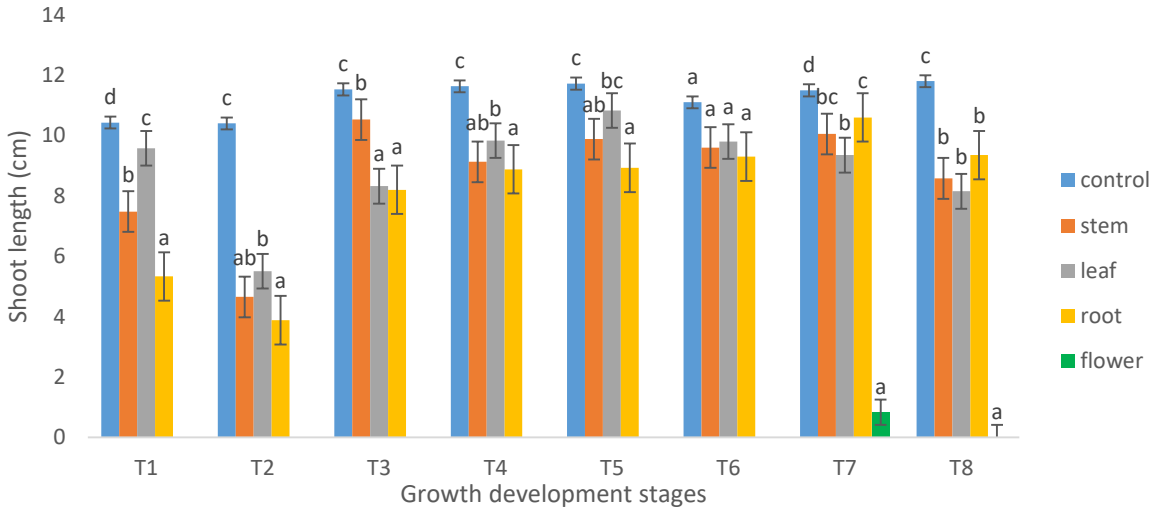

Figure 4.6. Effect of B. napus water extracts from (stem, root and leaf) collected at different times after germination (T1-T8) and flower collected at (T7 and T8) on the shoot length (cm) of Convolvulus arvensis 14 days after sowing. Bars with the same letter at different growth stages are not significantly different according to Duncan's multiple range test ($P < 0.05$).). Error bars represent the standard error of the mean. n = 192 for each treatment.

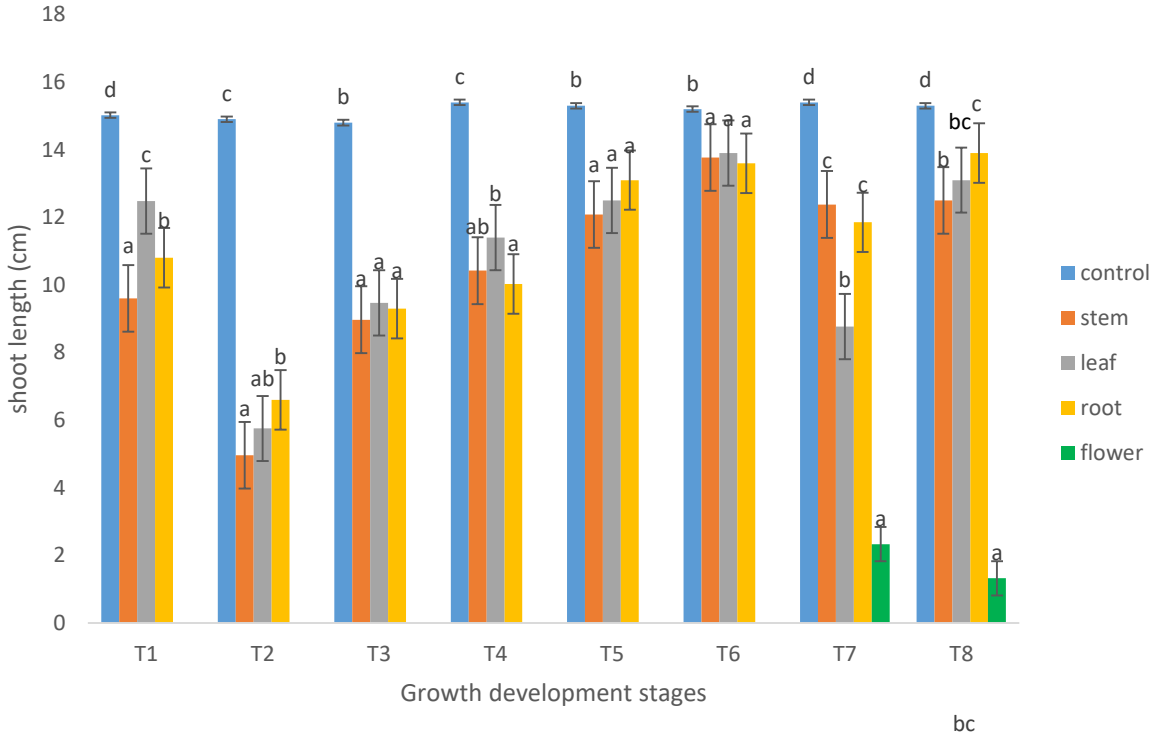

Figure 4.7. Effect of *B. napus* water extracts from (stem, root and leaf) collected at different times after germination (T1-T8) and flower collected at (T7 and T8) on the shoot length (cm) of *Sorghum halepense* 14 days after sowing. Bars with the same letter in each growth stage are not significantly different according to Duncan's multiple range test ($P < 0.05$). Error bars represent the standard error of the mean. n = 192 for each treatment.

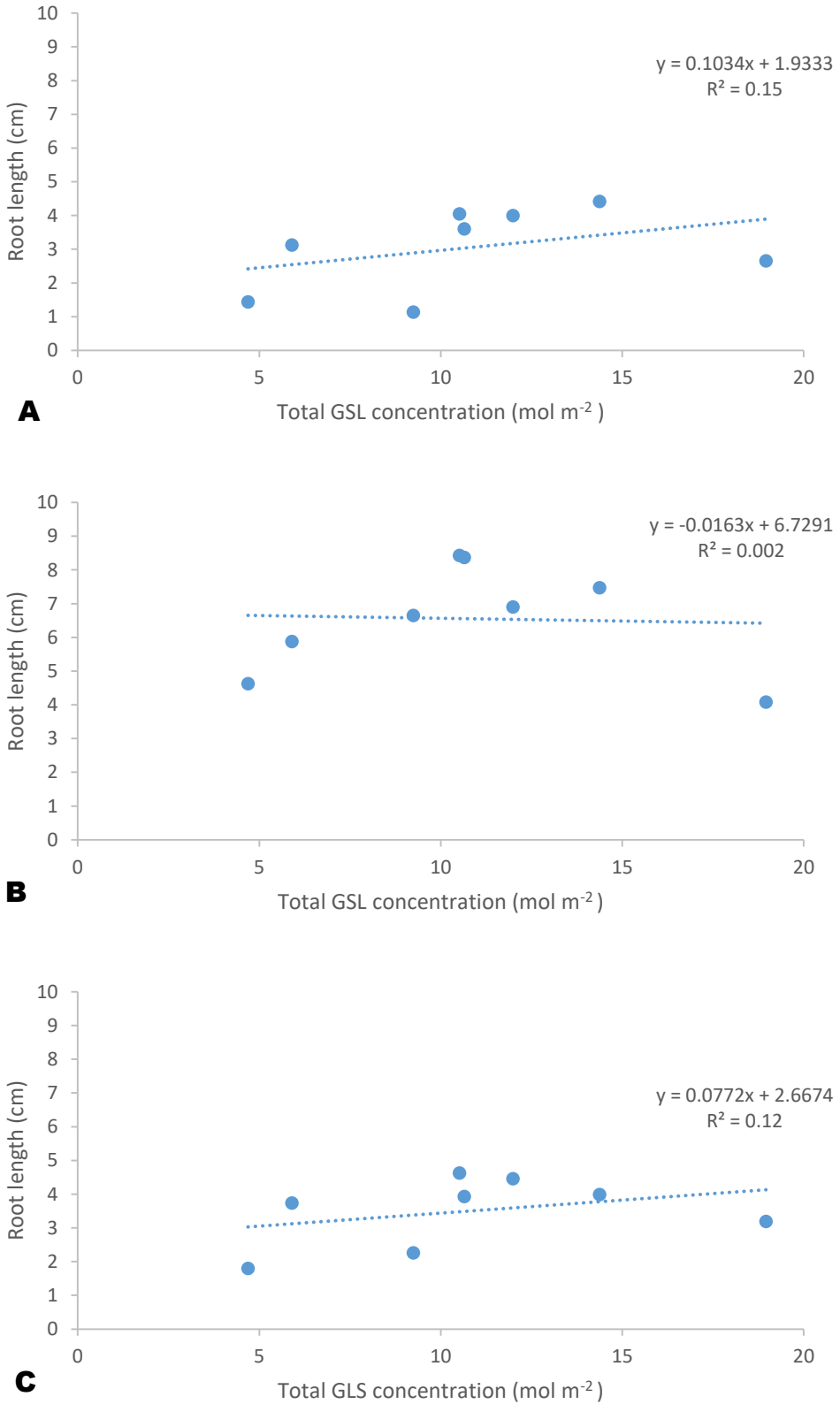

Figure 4.8. Relationship between root length (cm) of *Phalaris minor* (A), *Convolvulus arvensis* (B), *Sorghum halepense* (C) and Total glucosinolate (GSLs) concentrations during different development stages after 14 days.

4.4.1.3. Root length (cm)

The results of the data presented in figure 4.9, 4.10 and 4.11 and Anova variance table 4.3 revealed that water extract from all *B. napus* parts at all different development stages significantly ($P<0.001$) affected the root length of *P. minor* (Retz.), *C. arvensis* (L.) and *S. halepense* (L.) as compared with the control treatment. The root length of *P. minor* was completely inhibited when water extracts stems or leaves were applied at T1 and T8 respectively, and also flower water extract at T7 and T8 suppressed root length of *P. minor* completely. In T2 and T6, there was no significant effect was between suppression from stem or leaf extracts, however both treatments reduced *P. minor* significantly compared with the water extract made from root. Moreover, the stem extract at T3 and T4 was most effective on *P. minor* compared with extract from leaves and roots. Meanwhile, similar results were observed from using flower water extracts at T7 and T8 on *C. arvensis* root length, as it was inhibited significantly by up to 96.3% and 100% respectively (Figure 4.8). At the T1, water extract from stems was the most effective treatment on *C. arvensis* root length as compared with other treatments. At the T2 the difference between stem water extract treatments and water extract from roots was not significant. Roots extract at T5 and T6 appeared to be the most effective treatments in suppressing *C. arvensis* root length, followed by stem and leaf extracts.

Root length *S. halepense* was completely inhibited when treated with water extract from flowers at T7 and T8. Stem extract collected at T1-T5 appeared to be the most effective treatments in reducing *S. halepense* root length, with the next best treatment being water extract from leaves. Stem, leaf and root extract collected at T6 reduced *S. halepense* root length as compared with the control, but the difference between them was not significant. A weak realationship was found

between the root length of all three weed species *P. minor* (Retz.), *C. arvensis* (L.) and *S. halepense* (L.) and total GSL concentration (R^2 = 005, R^2 = 0.3 and R^2 = 0.57) respectively (Figure 4.12 A, B and C)

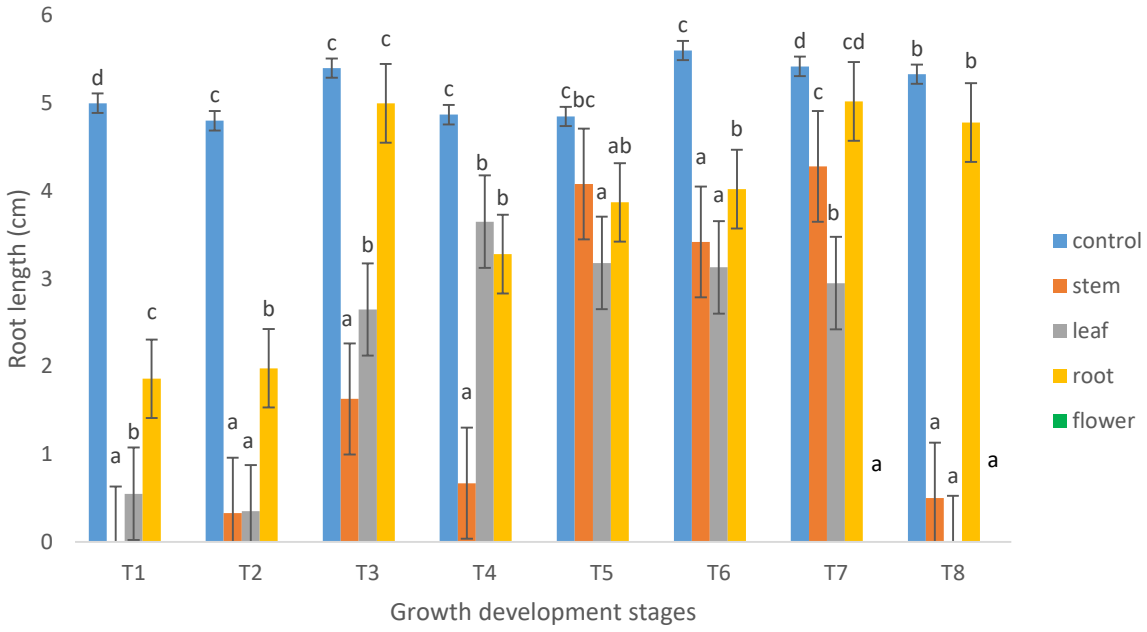

Figure 4.9. Effect of B. napus water extracts from (stem, root and leaf) collected at different times after germination (T1-T8) and flower collected at (T7 and T8) on the root length (cm) of Phalaris minor 14 days after sowing. Bars with the same letter in each growth stage are not significantly different according to Duncan's multiple range test (P < 0.05).). Error bars represent the standard error of the mean. n = 192 for each treatment.

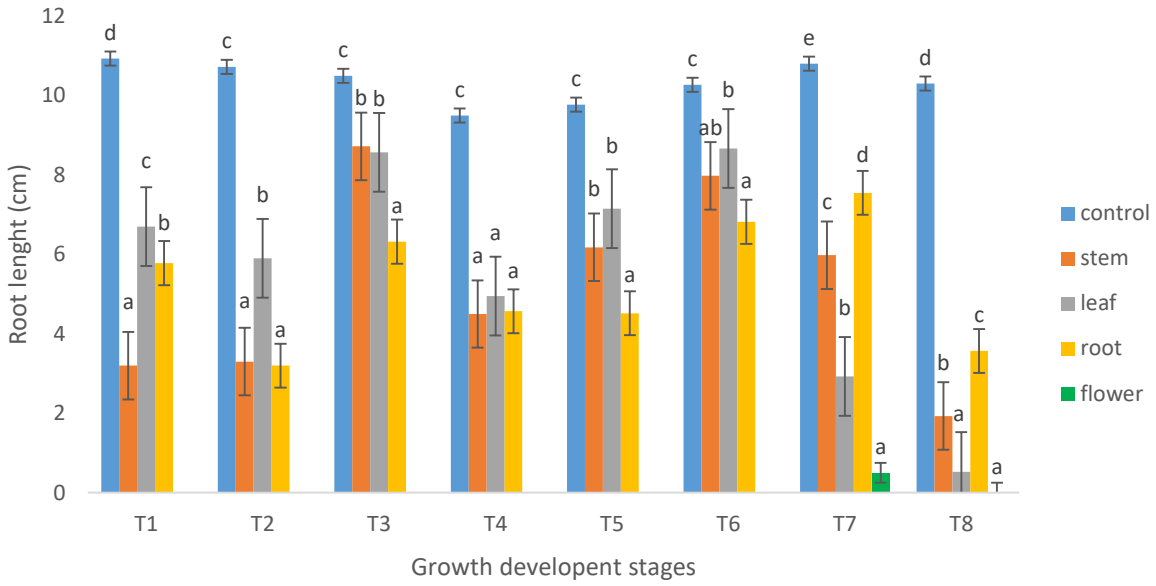

Figure 4.10. Effect of B. napus water extracts from (stem, root and leaf) collected at different times after germination (T1-T8) and flower collected at (T7 and T8) on the root length (cm) of Convolvulus arvensis 14 days after sowing. Bars with the same letter in each growth stage are not significantly different according to Duncan's multiple range test ($P < 0.05$).). Error bars represent the standard error of the mean. n = 192 for each treatment.

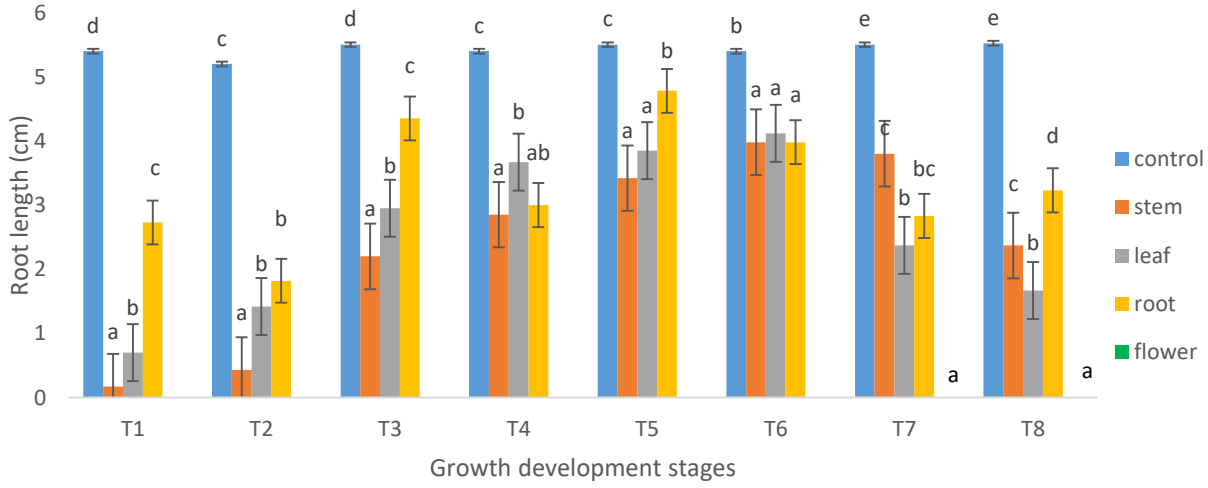

Figure 4.11. Effect of *B. napus* water extracts from (stem, root and leaf) collected at different times after germination (T1-T8) and flower collected at (T7 and T8) on the root length (cm) of *Sorghum halepense* 14 days after sowing. Bars with the same letter in each growth stage are not significantly different according to Duncan's multiple range test ($P < 0.05$).). Error bars represent the standard error of the mean. n = 192 for each treatment.

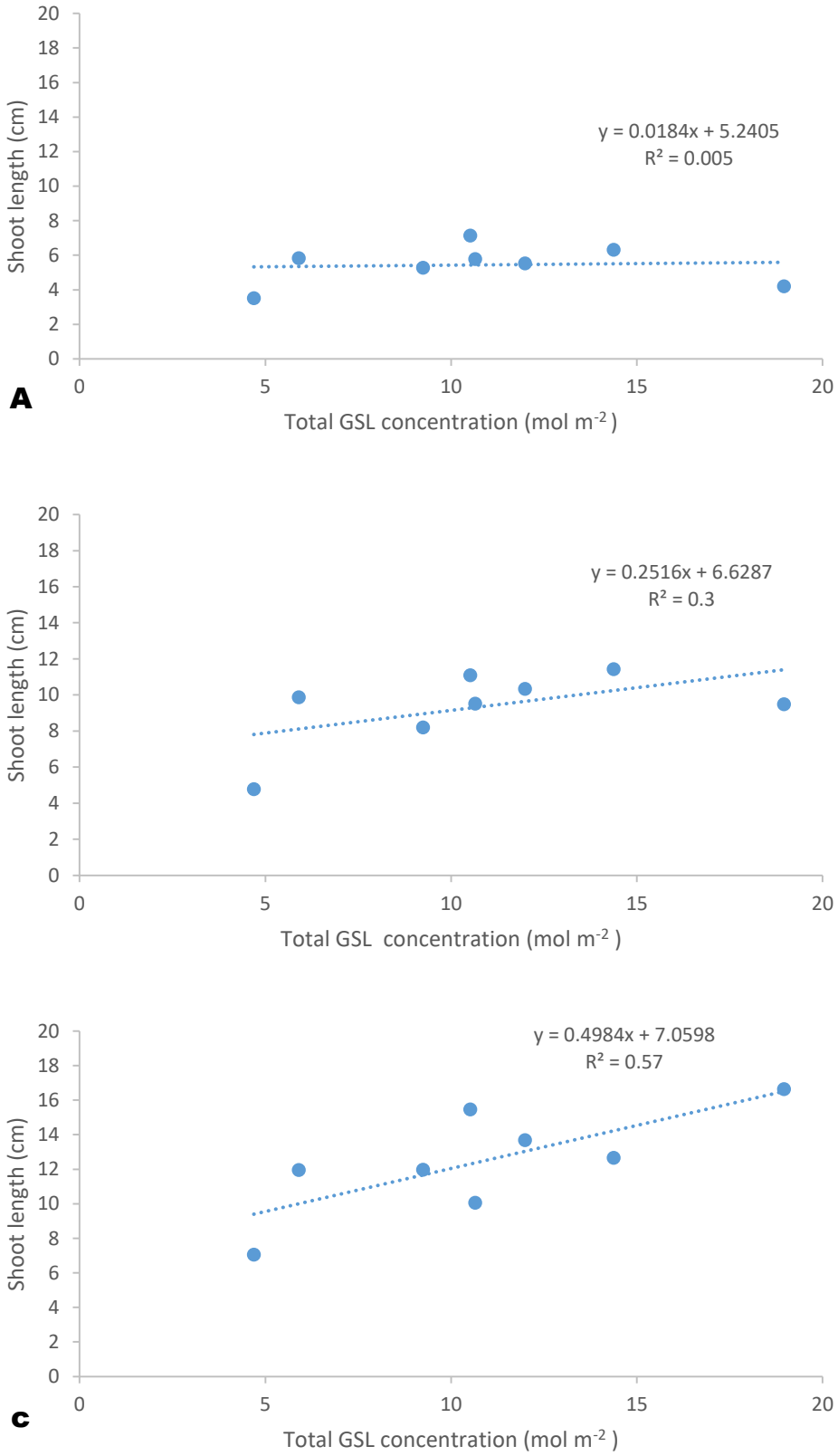

Figure 4.12. Relationship between shoot length (cm) of *Phalaris minor* (A), *Convolvulus arvensis* (B), *Sorghum halepense* (C) and Total glucosinolate (GSLs) concentrations during different development stages after 14 days.

4.4.1.4. Shoot fresh weight (mg)

The mean fresh weight of *P. minor shoots* (Retz.), *C. arvensis* (L.) and *S. halepense* (L.) are demonstrated in figure 4.13, 4.14 and 4.15 and Anova variance table 4.4. Water extracts from all *B. napus* parts collected at each development stage excluding T6 significantly ($p< 0.001$) reduced the fresh shoot weight of all three weed species as compared with the control. It was revealed that stem extracts collected at T1, T2, T3, T4 and T5 was the most effective treatment on *P. minor* as compared with other treatments where fresh weight of shoots were reduced by up to 29.25%, 36.7%, 67.5%, 27.2% and 32.1% respectively. Meanwhile, at T1 to T5, the difference between leaf water extract, and root water extract treatments was not significant (Figure 4.10). However, the flower extracts collected at T7 and the flower and leaf extracts collected at T8 appeared to be the most effective treatment in inhibiting *P. minor* fresh shoot weight (100% reduction) .

Flower water extract collected at T7 and T8 was significantly affected the fresh weight of *C. arvensis* and inhibited by up to 93.2% and 100% respectively as compared with the control (Figure 4.13). Additionally, the shoot fresh weight of *C. arvensis* was strongly inhibited by stem extract collected at T1, by up to 86.1%. Water extracts produced at T2, T5 and T6, from stem and root extracts were the most effective treatments on *the fresh weight of C. arvensis* shoots as compared with control and leaf extracts (Figure 4.13).

Shoot fresh weight of *S. halepense* was significantly ($P<0.001$) reduced by all *B. napus* water extracts collected as illustrated in Figure 4.14. At both growth stages T1 and T2, stem extract significantly inhibited *S. halepense* shoot fresh weight compared with other treatments. No significant differences were found between stems, leaves and roots at growth stages T3, T5, T6, T7 and T8. However, stem and root water extracts significantly reduced the *S. halepense* shoot fresh weight at

T4 compared with leaf extract. At growth stages T7 and T8, the flower extract appeared to be the most effective treatment in suppressing the fresh weight of *S. halepense* shoots showing significant (*P*<0.001) reductions of by up to 88.4% and 93.9% respectively. A weak realationship was found between the shoot fresh weight of all three weed species *P. minor* (Retz.), *C. arvensis* (L.) and *S. halepense* (L.) and total GSL concentration (R^2 = 0.05, R^2 = 0.0005 and R^2 = 0.11) respectively (Figure 4.16 A, B and C)

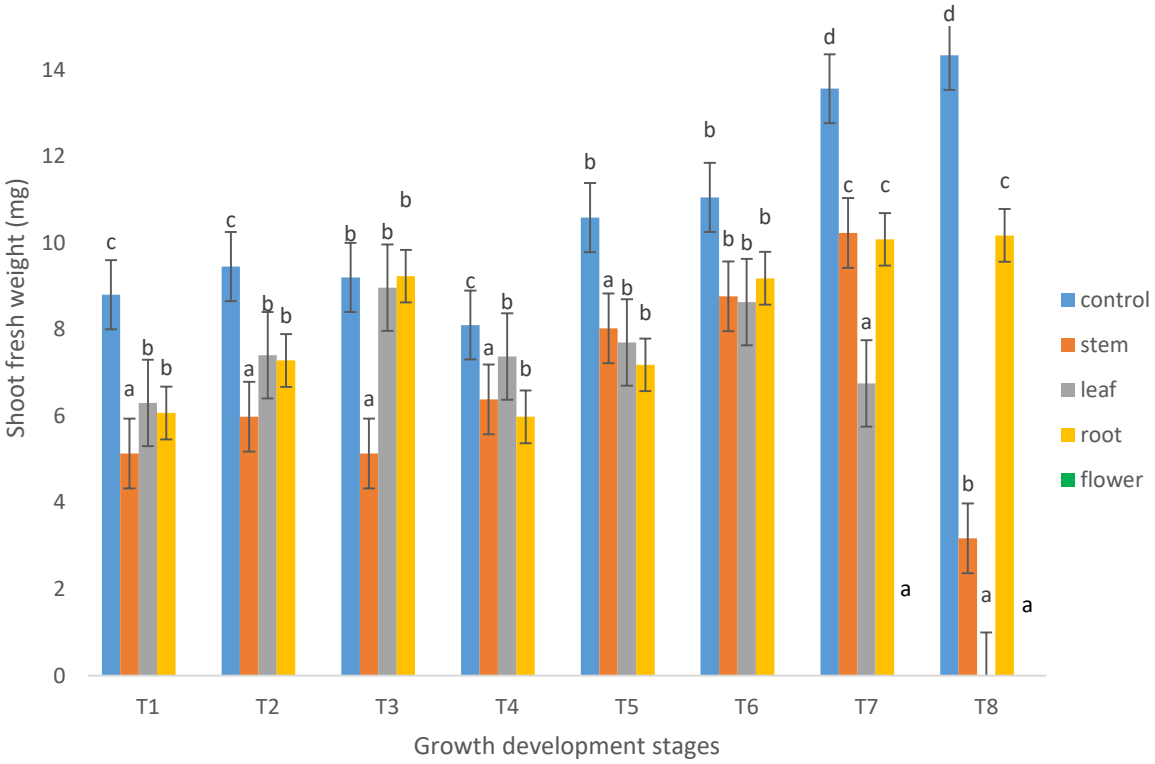

Figure 4.13. Effect of *B. napus* water extracts from (stem, root and leaf) collected at different times after germination (T1-T8) and flower collected at (T7 and T8) on the shoot fresh weight of *Phalaris minor* 14 days after sowing. Bars with the same letter in each growth stage are not significantly different according to Duncan's multiple range test (*P* < 0.05).). Error bars represent the standard error of the mean. n = 192 for each treatment.

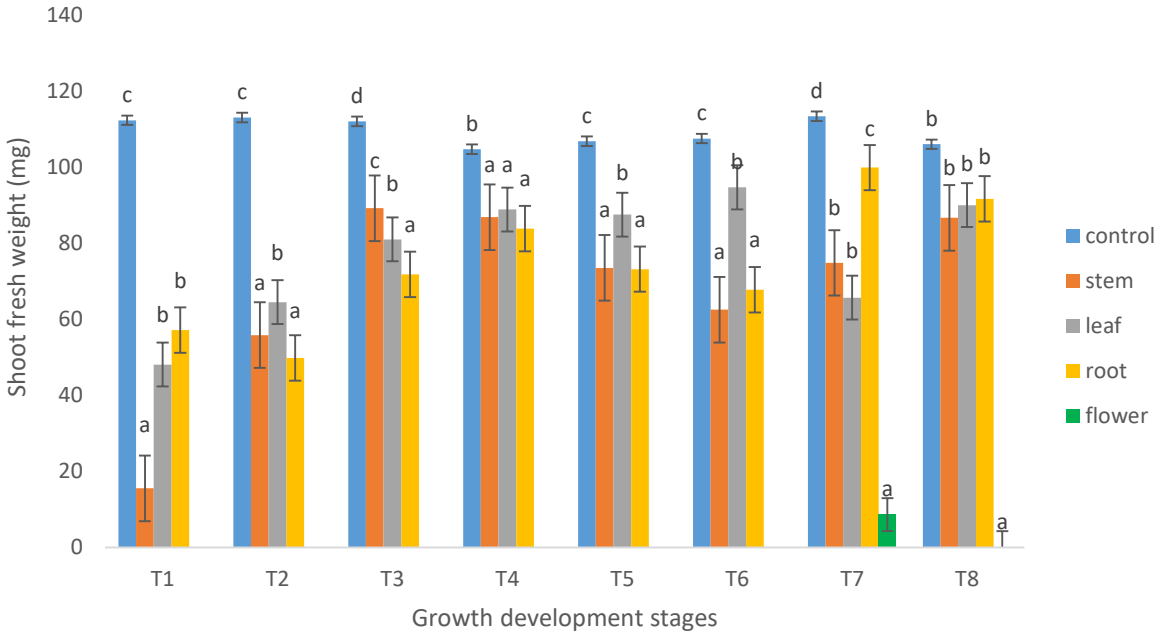

Figure 4.14. Effect of *B. napus* water extracts from (stem, root and leaf) collected at different times after germination (T1-T8) and flower collected at (T7 and T8) on the shoot fresh weight of *Convolvulus arvensis* 14 days after sowing. Bars with the same letter in each growth stage are not significantly different according to Duncan's multiple range test ($P < 0.05$).). Error bars represent the standard error of the mean. n = 192 for each treatment.

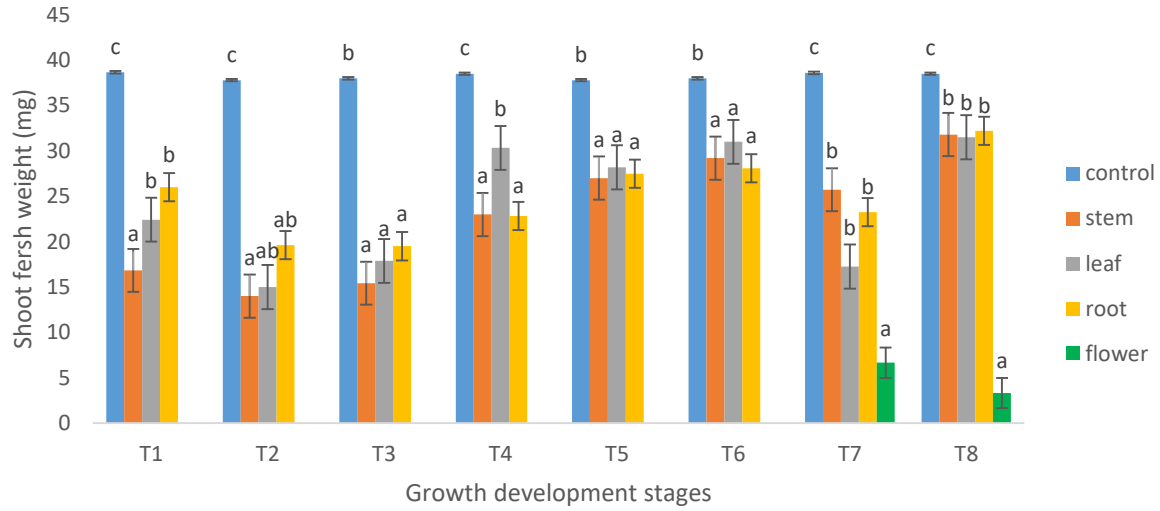

Figure 4.15. Effect of *B. napus* water extracts from (stem, root and leaf) collected at different times after germination (T1-T8) and flower collected at (T7 and T8) on the shoot fresh weight of *Sorghum halepense* 14 days after sowing. Bars with the same letter in each growth stage are not significantly different according to Duncan's multiple range test ($P < 0.05$).). Error bars represent the standard error of the mean. n = 192 for each treatment.

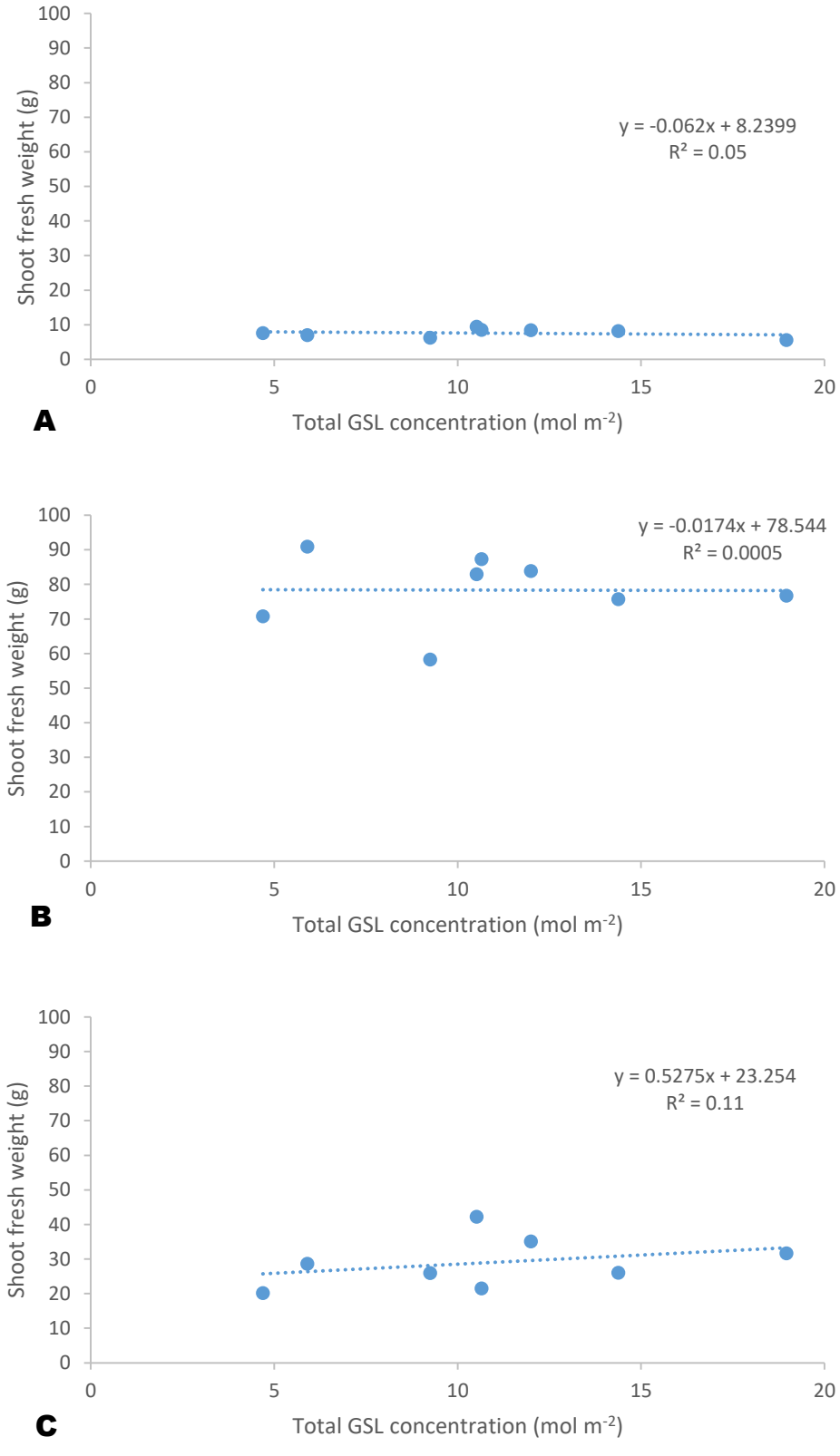

Figure 4.16. Relationship between shoot fresh weight (g) of *Phalaris minor* (A), *Convolvulus arvensis* (B), *Sorghum halepense* (C) and Total glucosinolate (GSLs) concentrations during different development stages after 14 days.

4.4.1.5. Root fresh weight (mg)

Phalaris minor (Retz.), *Convolvulus arvensis* (L.) and *Sorghum halepense* root fresh weight was significantly (*p<0.001*) affected by water extract from all parts of *B. napus* under different growth stages compared with control, as illustrated in Figures 4.17, 4.18 and 4.19 and Anova variance table 4.5. At growth stages T1 and T2, no significant differences were found between stem and leaf water extract, but both treatments highly affected *P. minor* root fresh weight and were significantly inhibited by 100% and 85.7%, 92.5% and 92.6% respectively (Figure 4.17). Stem extract collected at growth stage T3 and T4 was significantly reduced *P. minor* root fresh weight compared to the leaf and root extract. Moreover, at growth stages in T5 and T6, there was no significant difference revealed between stems, leaves and roots on their effect on *P. minor* root fresh weight, however all three treatments significantly reduced the root fresh weight of *P. minor* as compared with control.

Meanwhile, flower extracts collected at T7, and flower and leaf extract collected at T8 appeared to provide the most effective reduction of *P. minor* as compared with other treatments, with root fresh weight was suppressed completely.

Convolvulus arvensis root fresh weight was significantly (*p>0.001*) reduced by water extract collected at different plant development stages as shown in figure 4.18. Stem and leaf extracts collected at T1 and stem extracts at T2 were the most effective treatments in reducing root fresh weight of *C. arvensis* compared with other extracts and the control. Meanwhile, at growth stages T3, T4, T5 and T6, there were no significant differences between stem, leaf and root extracts and all three extracts were significantly reduced fresh root weight of *C. arvensis* in comparison with the control. The root fresh weight of *C. arvensis* was significantly affected by flower extract collected at T7 and reduced by 94.8%. Moreover, root fresh weight of *C. arvensis* was inhibited completely by flower extract collected at T8, and this was

followed by leaf extract. Flower extract collected at growth stages T7 and T8 were the most effective treatments in reducing root fresh weight of *S. halepense* (Figure 4.19). Moreover, at growth stage T1 stem extract completely inhibited the *S. halepense* root fresh weight. Similarly at T2, the greatest reduction of root fresh weight was observed on by applying stem extracts. A weak realationship was found between the root fresh weight of all three weed species *P. minor* (Retz.), *C. arvensis* (L.) and *S. halepense* (L.) and total GSL concentration (R^2 = 0.0002, R^2 = 0.24 and R^2 = 0.02) respectively (Figure 4.20 A, B and C)

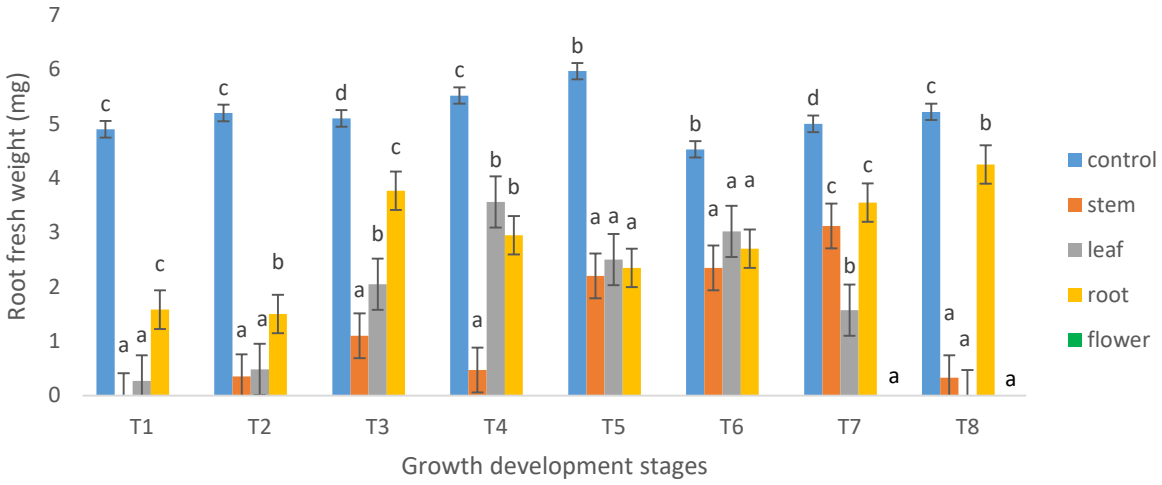

Figure 4.17. Effect of *B. napus* water extracts from (stem, root and leaf) collected at different times after germination (T1-T8) and flower collected at (T7 and T8) on the root fresh weight (mg) of *Phalaris minor* 14 days after sowing. Bars with the same letter in each growth stage are not significantly different according to Duncan's multiple range test ($P < 0.05$). Error bars represent the standard error of the mean. n = 192 for each treatment.

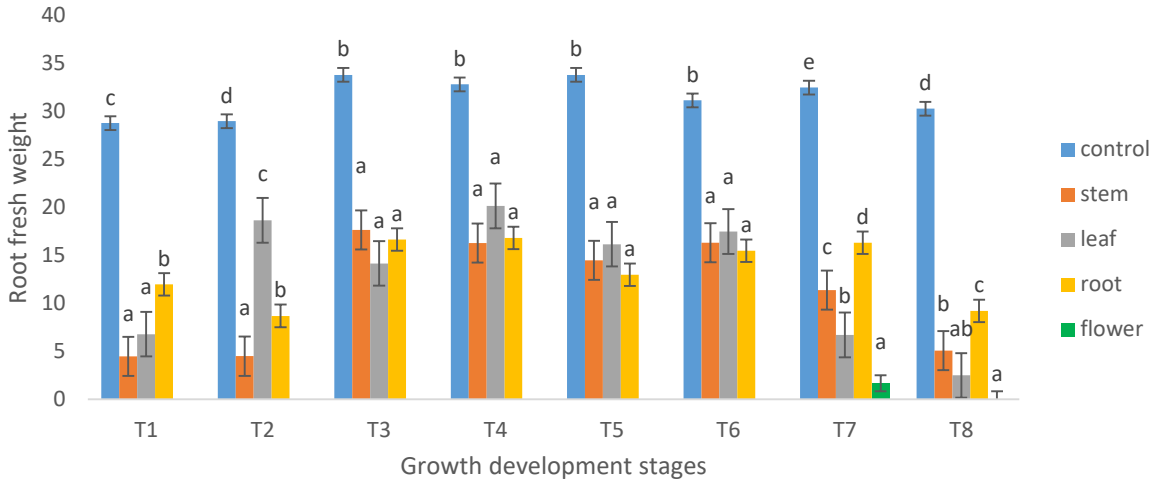

Figure 4.18. Effect of *B. napus* water extracts from (stem, root and leaf) collected at different times after germination (T1-T8) and flower collected at (T7 and T8) on the root fresh weight (mg) of *Convolvulus arvensis* 14 days after sowing. Bars with the same letter in each growth stage are not significantly different according to Duncan's multiple range test ($P < 0.05$). Error bars represent the standard error of the mean. n = 192 for each treatment.

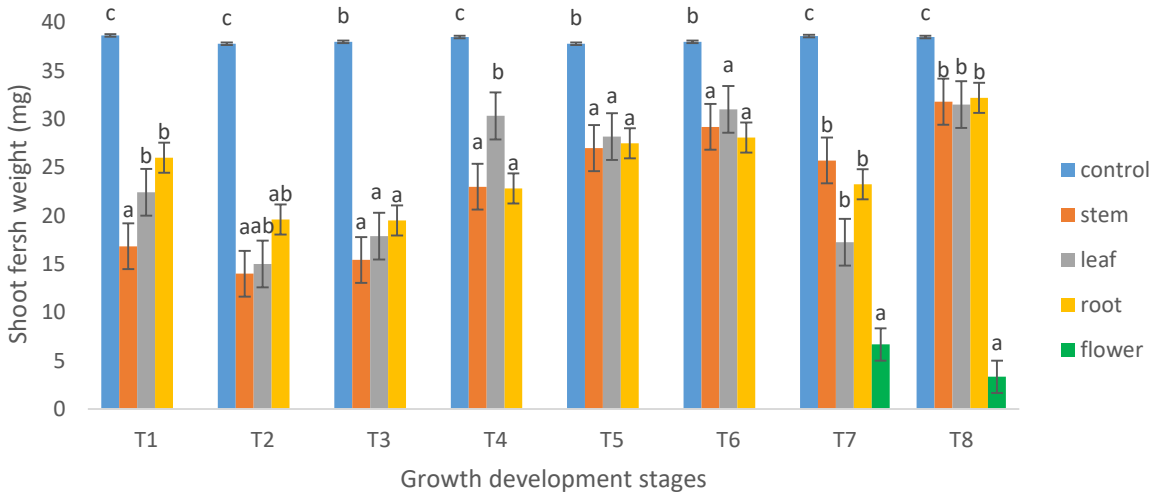

Figure 4.19. Effect of *B. napus* water extracts from (stem, root and leaf) collected at different times after germination (T1-T8) and flower collected at (T7 and T8) on the root fresh weight (mg) of *Sorghum halepense* 14 days after sowing. Bars with the same letter in each growth stage are not significantly different according to Duncan's multiple range test ($P < 0.05$). Error bars represent the standard error of the mean. n = 192 for each treatment.

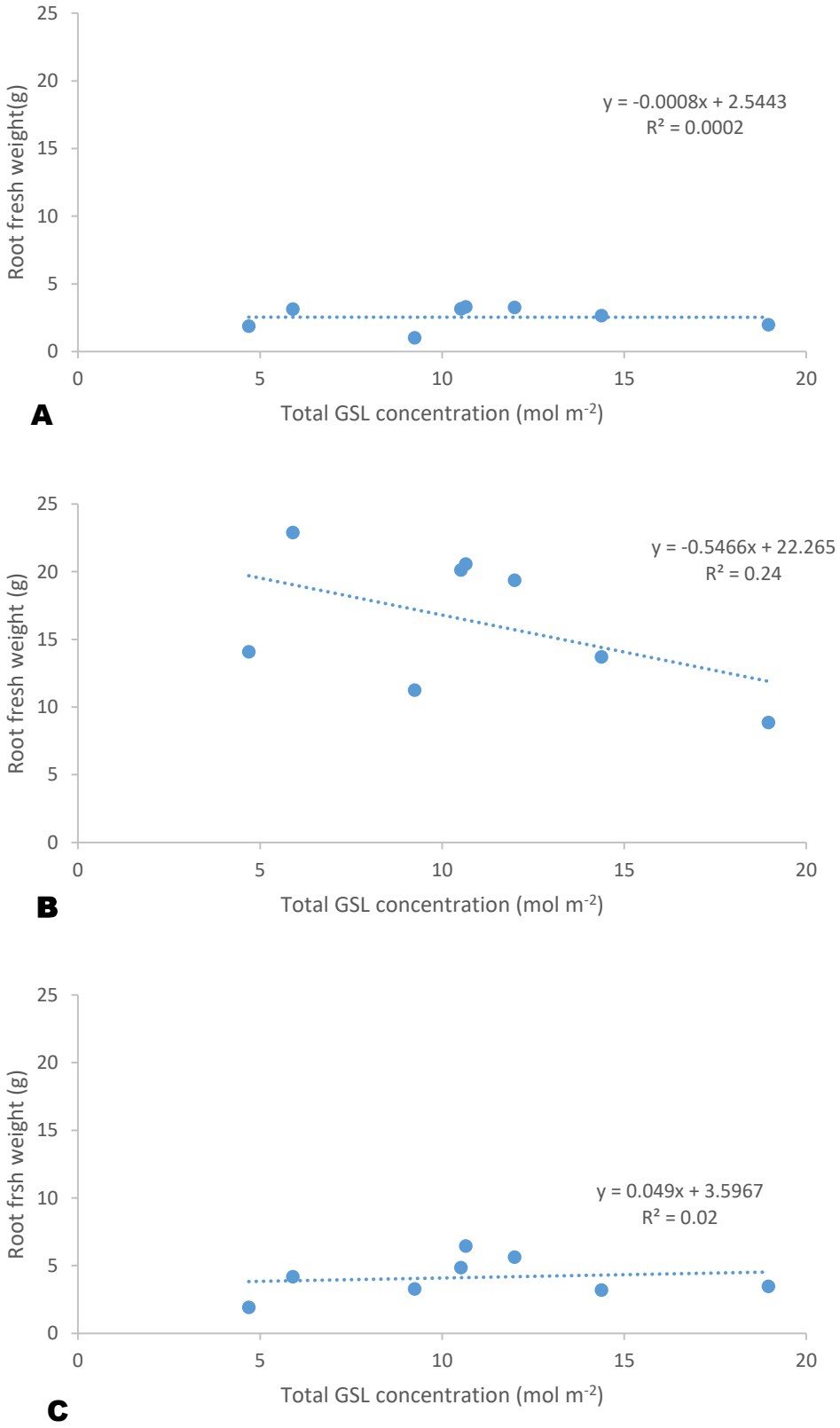

Figure 4.20. Relationship between root fresh weight (g) of *Phalaris minor* (A), *Convolvulus arvensis* (B), *Sorghum halepense* (C) and Total glucosinolate (GSLs) concentrations during different development stages after 14 days.

Table 4.1. Analysis of variance relating to the effect of water extract from different B napus parts collected at different growth development stages seed germination % of weed species

Source of variation	d.f.	Mean squares		
		Germination (%)		
		Phalaris minor	*Convolvulus arvensis*	*Sorghum halepense*
Plant development stages	7	621.35**	1269.94**	337.93**
Plant parts	3	62843.58**	38164.24**	43703.60**
Plant development stages x Plant parts	21	263.02**	586.06**	278.01**
Residual	160	31.15	26.61	33.83
Total	191			
CV%		14.8	12.9	18.8
SEM		5.581	5.159	5.816

**Significant at 1% probability level, *Significant at 5% probability level, NS = Non-significant

Table 4.2. Analysis of variance relating to the effect of water extract from different *B. napus* parts collected at different growth development stages on shoot length (cm) of weed species

Source of variation	d.f.	Mean squares		
		Shoot length		
		Phalaris minor	*Convolvulus arvensis*	*Sorghum halepense*
Plant development stages	7	31.5854**	105.7752**	217.900**
Plant parts	3	98.0017**	123.2010**	307.836**
Plant development stages x Plant parts	21	11.4716**	13.5432**	11.168**
Residual	160	0.8983	0.7265	2.734
Total	191			
CV%		17.4	9.1	13.3
SEM		0.9478	0.8523	1.653

**Significant at 1% probability level, *Significant at 5% probability level, NS = Non-significant

Table 4.3. Analysis of variance relating to the effect of water extract from different *B. napus* parts collected at different growth development stages on root length (cm) of weed species

Source of variation	d.f.	Mean squares		
		Root length		
		Phalaris minor	*Convolvulus arvensis*	*Sorghum halepense*
Plant development stages	7	36.0158**	62.0083**	24.5988**
Plant parts	3	80.6982**	355.3867**	111.1180**
Plant development stages x Plant parts	21	5.5649**	22.9085**	3.8503**
Residual	160	0.3604	0.6983	0.5309
Total	191			
CV%		19.7	12.8	20.8
SEM		0.6003	0.8356	0.7286

**Significant at 1% probability level, *Significant at 5% probability level, NS = Non-significant

Table 4.4. Analysis of variance relating to the effect of water extract from different *B. napus* parts collected at different growth development stages on shoot fresh weight (mg) of weed species

Source of variation	d.f.	Mean squares		
		Shoot fresh weight		
		Phalaris minor	*Convolvulus arvensis*	*Sorghum halepense*
Plant development stages	7	74.94**	11481**	1576.56**
Plant parts	3	231.729**	5584**	4767.58**
Plant development stages x Plant parts	21	32.556**	8366**	128.63**
Residual	160	2.414	3871	44.28
Total	191			
CV%		20.3	76.5	22.0
SEM		1.554	62.22	6.654

**Significant at 1% probability level, *Significant at 5% probability level, NS = Non-significant

Table 4.5. Analysis of variance relating to the effect of water extract from different *B. napus* parts collected at different growth development stages on root fresh weight (mg) of weed species

Source of variation	d.f.	Mean squares		
		Root fresh weight		
		Phalaris minor	*Convolvulus arvensis*	*Sorghum halepense*
Plant development stages	7	18.5771**	485.78**	46.496**
Plant parts	3	125.5564**	4254.15**	273.013**
Plant development stages x Plant parts	21	5.9051**	89.21**	9.935**
Residual	160	0.4120	16.02	1.030
Total	191			
CV%		24.2	23.6	23.5
SEM		0.6418	4.002	1.0147

**Significant at 1% probability level, *Significant at 5% probability level, NS = Non-significant

4.4.2. Individual and total glucosinolates concentrations

Ten glucosinolates (GSL) from different parts of *B. napus* were determined in the present study; glucoberin, progoitrin, epi- progoitrin, gluconapin, glucobrassinapin, glucoraphanin, glucobrassicin, 4OH glucobrassicin, neoglucobrassicin and gluconasturtiin.

4.4.2.1. Effect of different *B. napus* parts at different plant growth stages on the concentration of individual glucosinolates

There were great differences in the concentration of glucosinolates between the different plant parts at different plant growth stages. There was significant ($P< 0.001$) difference in the concentration of progoitrin, glucoraphanin, gluconapin and gluconasturtiin at different plant growth stages in *B. napus* stems (Figure 4.21).

The concentration of progoitrin was increased at growth stage T3, T7 and T8 and it was followed by treatment T6 and T5. However, progoitrin concentration at T1, T2 and T4 was significantly lower than other development stages. Glucoraphanin and gluconapin were not found at T1. The highest concentration of glucoraphanin was observed at T3 followed by T4. Gluconapin concentration was significantly higher at T5 than other development stages. Gluconasturtiin was one of the dominant glucosinolates in stem tissue and the concentration significantly ($P<0.001$) increased at plant development stage T1.

The lowest concentration of progoitrin in *B napus* roots was observed at plant development stages T1 and T2, however the concentration was significantly ($p<0.001$) higher in other development stages. The highest concentration of progoitrin was found at T5 and T6 followed by T8.

Glucoraphanin concentration in roots was low in all plant development stages. However, the concentration slightly increased with increasing plant age. A similar trend was found with gluconapin. Gluconasturtiin concentration was significantly (p<0.001) higher in *B. napus* roots at T1, than other stages, although the concentration increased again at flowering stage.

Low concentration of all glucosinolates; progoitrin, glucoraphanin, gluconapin and gluconasturtiin was observed in *B. napus* leaf extract at T1 , T2, and also in the T7 as no glucoraphanin and gluconasturtiin were found in this stage. Progoitrin concentration was found to be significantly higher in different plant development stages T3, T4 and T6, followed by T5 (Figure 4.22). Figure 4.23 demonstrates who the concentration of glucosinolates was affected by plant development stages in the flower extract. Progoitrin concentration increased in flower extract at both plant development stages T7 and T8. Also, gluconapin and gluconasturtiin concentration significantly increased at T8 in comparison to T7.

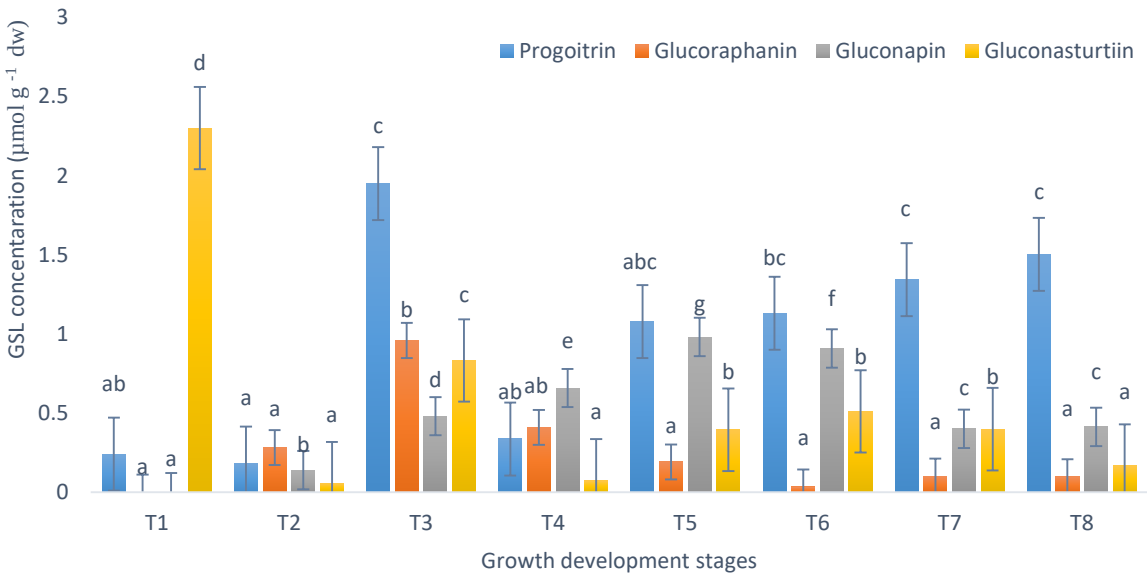

Figure 4.21. Glucosinolate concentration in the stems of B. napus at different times after germination (T1-T8). Bars with the same letter in each growth stage are not significantly different according to Duncan's multiple range test (P < 0.05). Error bars represent the standard error of the mean. n = 8 for each treatment.

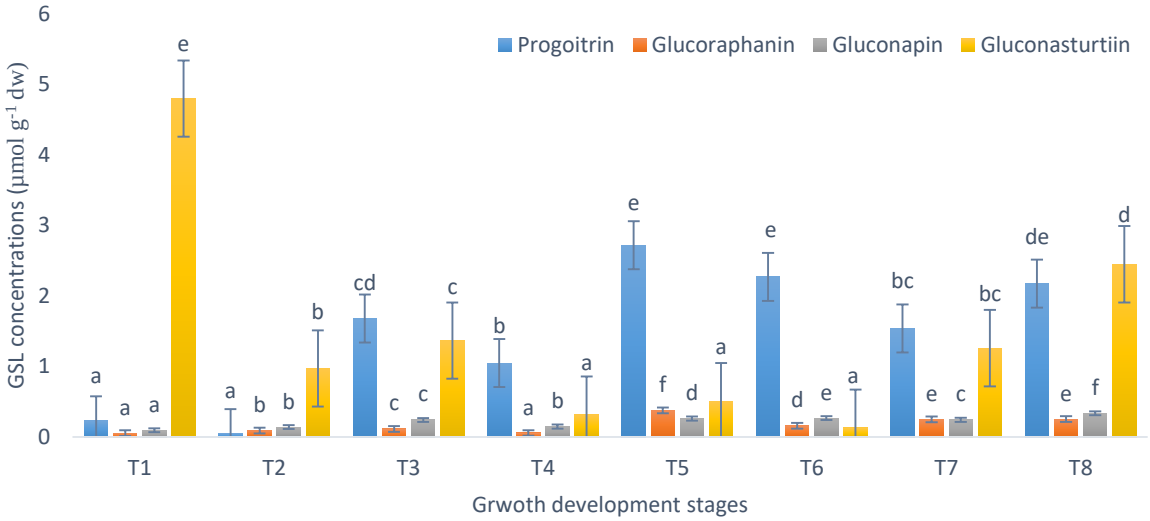

Figure 4.22. Glucosinolate concentration in the roots of *B. napus* at different times after germination (T1-T8). Bars with the same letter in each growth stage are not significantly different according to Duncan's multiple range test ($P < 0.05$). Error bars represent the standard error of the mean. n = 8 for each treatment.

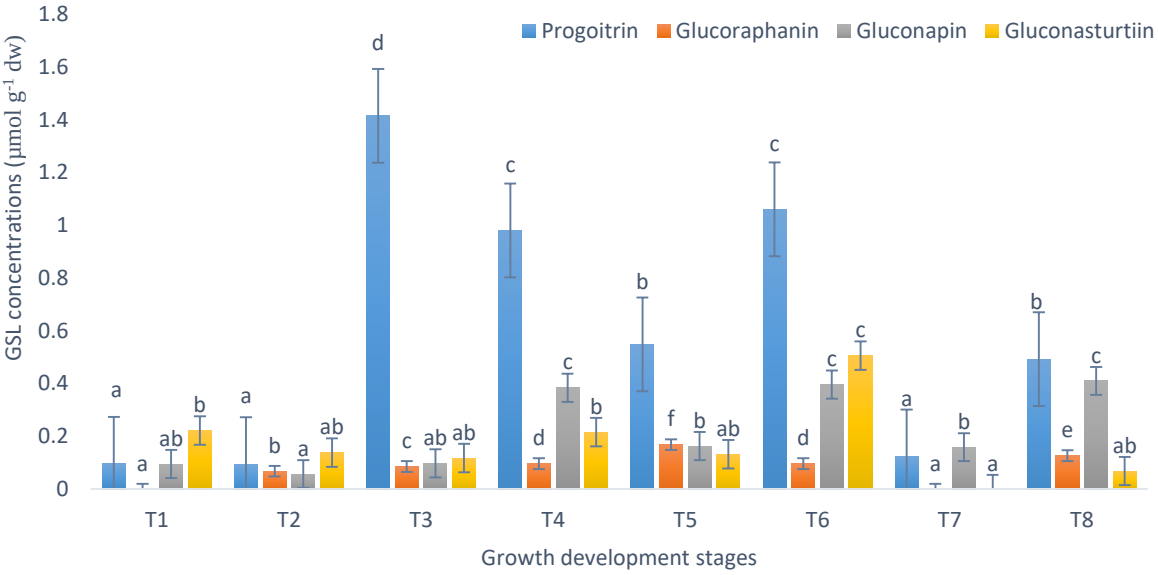

Figure 4.23. Glucosinolate concentration in the leaves of *B. napus* at different times after germination (T1-T8). Bars with the same letter in each growth stage are not significantly different according to Duncan's multiple range test ($P < 0.05$). Error bars represent the standard error of the mean. n = 8 for each treatment.

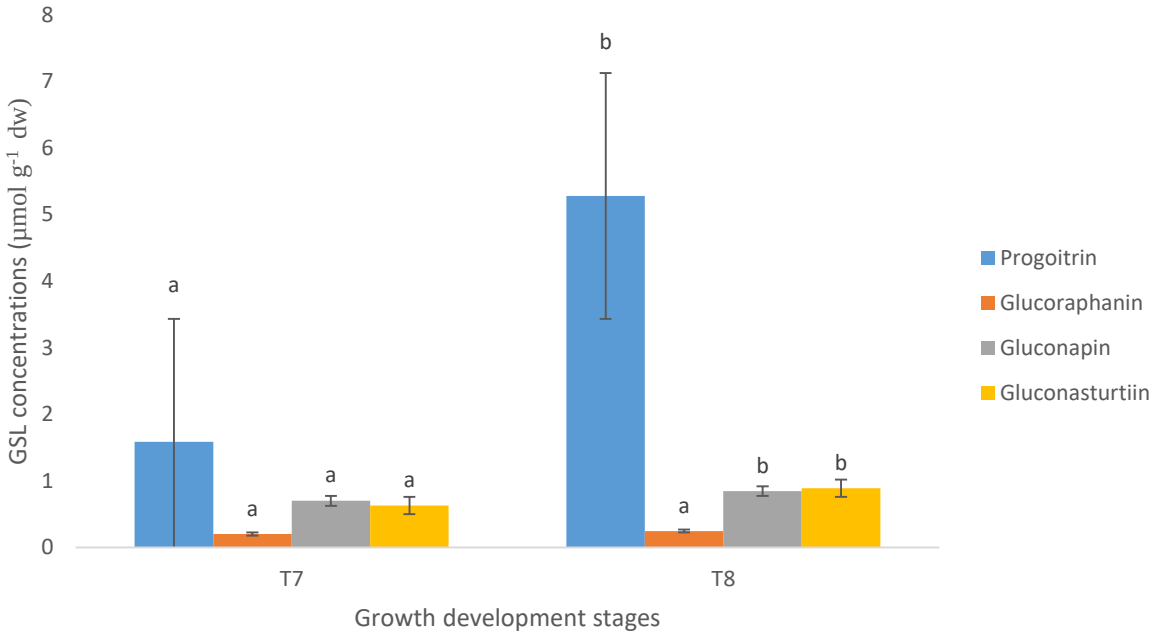

Figure 4.24. Glucosinolate concentration in the flowers of *B. napus* at different times after germination (T7-T8). Bars with the same letter in each growth stage are not significantly different according to Duncan's multiple range test ($P < 0.05$). Error bars represent the standard error of the mean. n = 8 for each treatment.

4.4.2.2. Effect of different *B. napus* parts at different plant growth stages on total glucosinolates concentration

There was significant ($p<0.001$) difference in the concentration of total glucosinolates in various parts of *Brassica* napus at different development stages (Figure 4.25). Total glucosinolates concentration in roots at development stages T1, T5 and T8 was significantly ($p<0.001$) higher as compared with concentration of total glucosinolates at T2, T3, T4, T6 and T7. Meanwhile, the concentration of total glucosinolates in stem extracts was significantly increased at T3 and T5 compared with other development stages. Whereas the total concentration of glucosinolates in the leaf extracts was low in early development and began to increase with plant development ages, while the total glucosinolate in the leaf extracts increased in mature development stages and the higher concentration was recorded at T7 and T8.

In general, the total and individual GSL concentration in flowers extract was significantly higher (p > 0.001) than other parts. Also the total GSL concentration in roots was significantly higher (P > 0.001) than GSL concentration in stems and leaves (Figure 4.25).

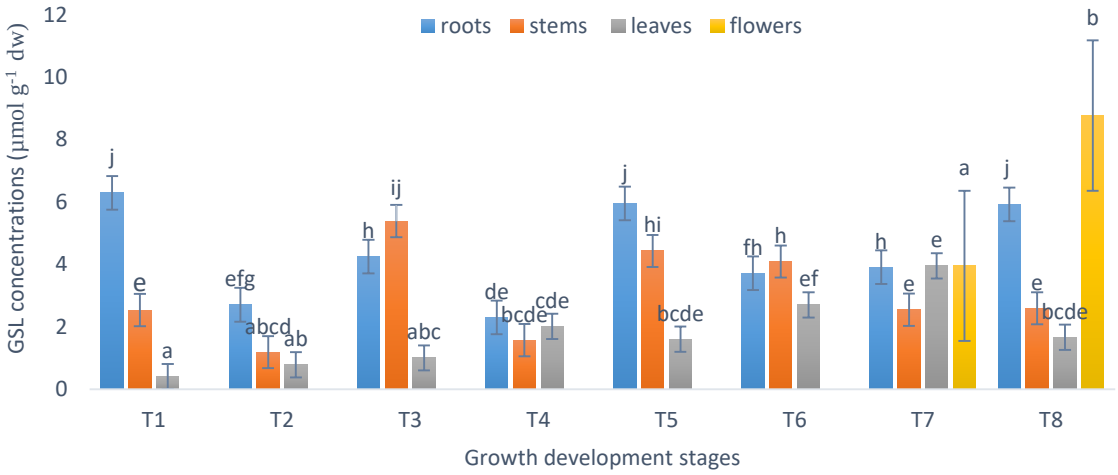

Figure 4.25. Total glucosinolate concentration in the *B. napus* parts at different times after germination (T1-T8). Bars with the same letter in each growth stage are not significantly different according to Duncan's multiple range test (P < 0.05). Error bars represent the standard error of the mean. n = 8 for each treatment.

4.4.3. Effect of different *B. napus* parts at different plant growth stages on myrosinase enzyme activity

There were significant (P <0.05) differences in the activity of myrosinase between different *B. napus* tissues and plant development stages (Figure 4.26). Myrosinase activity in root extracts at T2 was significantly (p=0.007) higher than other plant development stages, with the enzyme activity reducing with increasing plant age. The myrosinase activity in stem extracts was significantly (p<0.001) highest at T2, T3, T4 and T5, but was found to reduce at later development stages. In comparison, the levels of myrosinase activity in leaf extracts were significantly than root or stem extracts. The highest enzyme activity in leaf extracts was determined at T4 and T5.

Flower extract, myrosinase activity was not found to be significantly different between stages T7 and T8. However, the enzyme activity was the highest in flower extracts when compared with the enzyme activity root, stem and leaf extracts.

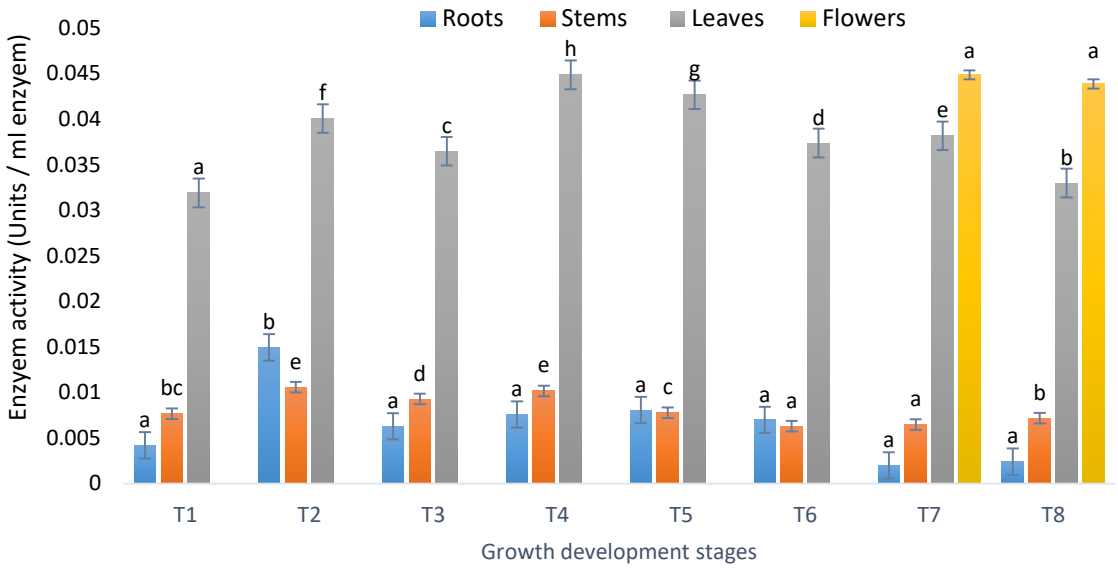

Figure 4.26. Myrosinase activity in the *B. napus* parts at different times after germination (T1-T8). Bars with the same letter in each growth stage are not significantly different according to Duncan's multiple range test ($P < 0.05$). Error bars represent the standard error of the mean. n = 8 for each treatment.

4.5. Discussion

4.5.1 Weeds species germination and seedling growth

In the last three decades, the effect of water extracts from crop plants such as *B. napus* on weed species germination and seedling growth has received additional attention by researchers (Tawaha and Turk, 2003; Wakjira *et al*., 2005; Uremis *et al*., 2009; Toosi and Baki, 2012; Modhej *et al*., 2013; Al-Sherif *et al*., 2013; Walsh *et al*., 2014). This study of water extracts from different plant parts at different development stages has demonstrated variability in germination and seedling growth of *P. minor* (Retz.), *C. arvensis* (L.) and *S. halepense*. As presented in

section 4.4.1.1, the germination of all three weed species significantly was reduced by applying water extract from all plant parts and at all plant development stages compared to the control. However, inhibition of weed species germination depended on the type of extract used. The results obtained in this present study are in congruent with other studies that documented variation in the suppressive effect of allelopathic plants depending on the type of plant tissue selected (Chon and Kim, 2002; Turk and Tawaha, 2002; Turk and Tawaha; 2003). For example, Turk and Tawaha (2003), found that leaf water extracts from *Brassica nigra* were the most effective on wild barley (*Hordeum spontaneum*). Additionally, Tollsten and Bergstrom (1988) observed that allyl-isothiocyanates (ITC) isolated from *B. nigra* residues suppressed the germination of grass species in a natural grassland, Also found that the *Brassica alba* can produce a Benzyl-ITC, who was able to inhibited seed germination and seedling growth of *Abutilon theophrast* (velvetleaf), *Senna obtusifolia* L., *Cassia obtusifolia* L. (sicklepod), and sorghum (*Sorghum bicolor* (L.).

The variation between the effect on plant development stages and on germination of weed species could be due the differences among individual and total glucosinolates concentrations found in *B. napus* at different plant development stages. These results match with data observed by Brown *et al*., (2003) and Redovniković *et al*., (2008), who reported differences between the glucosinolate profiles and their concentrations in the different plant parts and during *B. napus* developmental stages. Meanwhile, no germination was recorded when flower water extract collected at T7 and T8, was applied and the reduction was 100%. These results are in agreement with that reported by (Abdel-Farid *et al*., 2014), who found that canola seeds were very sensitive to flower water extract of *Calotropis procera* as compared with the other parts.

In general, all weed species germination had similar sensitivity to stem, leaf and root extracts at T4 and T5. The allopathic effect caused by extracts from *B. napus* stems, leaves and roots appears to be a consequence of the bioactivity of same chemical compounds produced by hydrolysis of glucosinolates (Angus *et al*., 1994; Buskov *et al*., 2002; Bellostas *et al*., 2007). On the other hand, *C. arvensis* germination was completely suppressed by applying flower extract at plant developmental stages during T8 and the redaction was 100% and also at T7, it was the most effective as well as with *S. halepense* germination. These results may be due to high concentrations of progoitrin, total glucosinolates and the allyl-isothiocyanates (ITC) product from flower water extract through GSLs hydrolysis by enzyme myrosinase during the flowering stage. These findings are in line with those reported by Peterson *et al*., (2001), Malik *et al*., (2010) and Modhej *et al*., (2013). They reported that the inhibition in weed germination by *Brassica spp*. was probably due to isothiocyonamatic compounds which had high ability to suppress the seed germination. In additional, Malik, 2009; have been found the highest level of GSLs in flower of Wild radish (*Raphanus raphanistrum*), and the most dominant GSLs were glucotropaeolin, glucobrassicin and glucoraphanin.

All dominant glucosinolates present in this study are degraded to isothiocyanates, which are linked with weed inhibition (Song *et al*., 2005; *Uremis et al., 2009*; Malik *et al*., 2010). Furthermore, *Sorghum halepense* germination appears to be the most sensitive species for all water extracts from various plant tissues.

4.5.2. Weed species shoot and root length

Observations of applying water extracts from all *B. napus* parts during all plant development stages on weed species (Figures 4.5 to 4.11) confirmed that shoot length and root length of weed species were affected by the application of all water extracts. The reduction of *P. minor* shoot length was significantly greater with

applying stem extracts during different growth development stages (T1 to T6). The reduction in weed species may refer to the phytotoxicity effect of companion between all glucosinolate observed in *B. napus* tissue. This result confirms the findings of Chung and Miller (1995), Turk and Tawaha (2003), who document that a mixture of water extracts from all black mustard parts significantly suppressed the shoot length of weed species as compared with the control. Moreover, *P. minor* shoot length appeared to be most sensitive to flower extract during plant development stage T7, and also leaf and flower extracts at T8. This finding might be due to the combination between progoitrin and gluconapin because, as the results show in figure 4.23, the glucoraphanin and gluconasturtiin disappeared in leaf extracts during T7. Overall, an almost identical trend was found with the reduction of *C. arvensis* and *S. halepense* shoot length during different plant development stages by applying water extract from all plant parts (Figures 4.6 and 4.7).

Furthermore, root length of all three weed species appeared to be more sensitive to GSLs observed in this study as compared with weed species shoot length, (Figures 4.9, 4.10 and 4.11). Therefore, the glucosinolates determined in this study may have herbicidal activity potential for weeds through affecting the roots weed species. These observations are in agreement with earlier results reporting that plant water extract had more toxic effects on weeds' root length than on weeds' shoot length (Chung and Miller, 1995; Tawaha and Turk, 2003; Turk and Tawaha, 2003; Turk *et al*. 2005). These outcomes might be due to the roots being the first to imbibe the allelochemicals from the water extracts (Jenning and Nelson, 2002; Turk and Tawaha 2002). In addition to the root inhibition by different water extract, weed root morphology was deformed, such as root twisting, compared with the control (Jenning and Nelson, 2002; Matloob *et al*., 2010).

4.5.3. Weed species shoot and root fresh weight

In the present study, the fresh weight of the shoots from all three weed species was significantly suppressed by applying water extracts from all *B. napus* parts during different plant growth development (Figures 4.13 to 4.19). Stem extract seemed to be the most effective treatment on the fresh weight of *P. minor* shoots during development stages T1 to T5. However, there was higher redaction on fresh weight during T7 and T8 caused by leaf as compared to the stem and root extracts. Also, because of no germination recorded for *P. minor* when treated by flower extract under T7 and T8, thus no shoot length and shoot fresh weight will be recorded. Furthermore, the degree of inhibition of shoot fresh weight of *C. arvensis* with applying stem extract during T1 was largely dependent on the shoot length.

These outcomes may be due to the reductions happened in shoot length. Mainly there were no significant differences between water extract from stems, leaves and roots to *S. halepense* shoot weight during plant growth stages T2, T3, T5, T6 and T7. However, during T1 the stem extracts exhibit stronger phytotoxicity to *S. halepense* shoot weight. These findings are in line with those reported by (Vene *et al*., 1987; *Uremis et al., 2009*; Yasumoto *et al*., 2010 and 2011), who documented that *Brassica spp*. had phytotoxicity on the growth of the following crops; they demonstrated that mixing *Brassica spp*. biomass into soil reduced the plant density in followed crops.

In Figures 4.17, 4.18, 4.19, the significant reduction in all weed species' root fresh weight caused by water extracts from all *B. napus* parts during different plant development stages were probably because of allelopathic effects on root length as previously described. These findings are in line with other studies reporting the inhibitory effects of water extracts of allelopathic *Brassica spp*. (Al-Khatib *et al*., 1997; Krishana *et al*., 1998). Flower extracts from *B. napus* reduced the root fresh

weight of all three weed species significantly (Abdel-Farid *et al*., 2014). Different parts from the *B. napus* show different effects on weed fresh weight (Tanveer *et al*., 2010; Chandra and Mali, 2012; Abdel-Farid *et al*., 2014), this may refer to the profile of allelochemicals found in different plant parts (Cheema *et al*., 2007; Sisodia and Siddiqui, 2010).

4.5.4. Individual, total glucosinolates and myrosinase activity

The *Brassica napus* parts (stems, leaves, roots and flowers) studied, revealed variability in concentration and type of individual glucosinolates between the different parts and within the parts during different plant development stages. Also, the total concentration of glucosinolates was affected by *Brassica napus* parts (stems, leaves, roots and flowers) during different plant development stages. These results are in line with those stated in literature (McGregor, 1988; Kirkegaard & Sarwar, 1998; Lambdon et al., 2003; Bellostas *et al*., 2007). Ten glucosinolates were determined in different parts of *B. napus* in present study; Glucoberin, progoitrin, epi- progoitrin, gluconapin, glucobrassinapin, glucoraphanin, Glucobrassicin, 4OH glucobrassicin, neoglucobrassicin and gluconasturtiin. The major glucosinolates based on concentrations were progoitrin, glucoraphanin, gluconapin and gluconasturtiin.

In general, from accounting the total glucosinolates, the highest total glucosinolates were observed during plant development stages T7 and T8 and the total glucosinolates in flowers were significantly different as compared with the other parts. This means that the allelopathic effect observed for flower water extract (Chapter 2 and 3) was resulting from the ITC associated with glucosinolates in this water extracts. This was well supported by results from experiments (Chapter 5) in which those glucosinolates were shown to be greatly toxic to weed species

germination and seedling growth. These findings may due to high concentrations of the dominant glucosinolate (progoitrin) found in the flower tissues. Recently, a similar allelopathic effect of flower extract has been described by Abdel-Farid *et al*. (2014), who found that the glucosinolates in flower extracts showed high toxicity to weed germination and their growth. Additionally, stem extracts show a high toxicity to weed germination and seedling growth under different plant growth stages.

In general, *Brassica spp*. members are using the glucosinolate- myrosinase system as a defence system against antagonists (Bennett and Wallsgrove, 1994; Wittstock and Gershenzon, 2002). In the present study, the data revealed significant differences in myrosinase activity between different plant parts during different plant growth development stages*,* despite the myrosinase activity in leaf extracts being significantly higher than other plant parts during development stages T1 to T6 (Figure 4.26). Furthermore, water extracts from other plant parts in during numerous stages of plant development, such as stem extract, were significantly more effective on weed species germination and seedling growth, or there was no significant differences between them. Also, during plant development stages T7 and T8, myrosinase activity in flower and leaf extracts were high, although flower extract was more effective as compared with leaf extracts. These results were in line with the observation reported by Hansen (2011), who found that although the differences in soil myrosinase activity were detected among the samples, there was no positive correlation between soil myrosinase activity and glucosinolates concentrations released after incorporation of freeze-dried mustard leaves.

4.6. Conclusion

The results of this experiment revealed that water extracts from all parts of *Brassica napus*, collected at different development stages, significantly (p<0.001) inhibited seed germination % of all three weed species. No germination recorded for *P. minor*

when treated by flower extract under T7 and T8, thus no shoot length and shoot fresh weight will be recorded.

The reduction of *P. minor* shoot length was significantly greater with applying stem extracts during different growth development stages (T1 to T6). Additionally, *P. minor* shoot length appeared to be most sensitive to flower extract during plant development stage T7, and also leaf and flower extracts at T8.

Root length of all three weed species seemed to be more sensitive to GSLs observed in this study as compared with weed species shoot length. Stem extract was the most effective treatment on the fresh weight of *P. minor* shoots during development stages T1 to T5. The highest total glucosinolates were observed during plant development stages T7 and T8 and the total glucosinolates in flowers were significantly different as compared with the other parts. Furthermore, it was found a significant differences in myrosinase activity between different plant parts during different plant growth development stages, despite the myrosinase activity in leaf extracts being significantly higher than other plant parts during development stages T1 to T6.

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Chapter 5

5. Effect of pure glucosinolates and myrosinase enzyme on weed species germination and seedling

5.1. Introduction

Glucosinolates (GSLs) are one group of the secondary plant metabolites found in *Brassica spp*. Hydrolyses of GSLs by the enzyme myrosinase results in an array of volatile and biocidal compounds such as isothiocyanates, which have the ability to suppress weed seeds (Brown and Morra, 1997; Al-Turki and Dick, 2003).

More than 130 individual glucosinolates have been observed in several plant families (Fahey *et al*., 2001; Agerbirk and Olsen, 2012; Rameeh, 2015). Researchers have reported that glucosinolates cannot be effective without hydrolysis by effective myrosinases enzyme (thioglucosidase glucohydrolase; EC 3.2.3.1). These chemical compounds may be toxic to a variety of organisms such as weed species and fungal pathogens (Halkier and Gerahenzon, 2006; Jafarieyazdi and Javidar, 2011).

Several studies carried out in the laboratory and glasshouse demonstrated that the hydrolysis products of glucosinolates, in addition to brassica water extracts and brassica residues containing these chemical compounds like isothiocyanate, reduce weed germination and seedling growth and development (Mason-Sedun *et al*, 1986; Bialy *et al*., 1990; Brown and Morra, 1997; Al-Turki and Dick, 2003; Webater, 2005; Norsworthy *et al*., 2006; Bangarwa *et al*., 2010; Bangarwa and Norsworthy, 2014).

Also, research recommends that using Brassicaceae members as a cover crop may be phytotoxic to numerous crop seeds. For example, an experiment conducted in the glasshouse has shown that chemical compounds produced by brassica cover crops (isothiocyanates) have the ability to suppress shoot density and shoot biomass of purple nutsedge (*Cyperus rotundus* L.) and yellow nutsedge (*Cyperus esculentus*) (Norsworthy *et al*., 2006).

Petersen *et al*. (2001), suggest that weed inhibition in the field was possible because of the high concentration of ITCs found in turnip rape mulch. Also, they reported that Isothiocyanates strongly affect the germination of the tested species and possibly interact with weed seeds in the soil solution and as vapour in soil pores.

5.1.1. Aim

The present study was undertaken to evaluate the effect of pure glucosinolates in the presence and absence of myrosinase enzyme on seed germination and seedling growth of *Phalaris minor* (Retz.), *Convolvulus arvensis* (L.) and *Sorghum halepense.*

5.1.2. Hypotheses

Seed germination and seedling growth of weed species are not affected by pure glucosinolates in the presence and absence of myrosinase enzyme.

5.2. Material and methods

5.2.1. Chemicals

Glucoraphanin (R-(-) - Glucoraphanin potassium salt, ≥99%) and gluconasturtiin (Phenethyl glucosinolate potassium salt, ≥97%) were purchased from Santa Cruz Biotechnology, Inc. Texas, USA. Gluconapin (Gluconapin potassium salt ≥98%), was purchased from (Bio Sciences, Creative Dynamics, Inc. USA). Myrosinase enzyme (thioglucosidase glucohydrolase; EC 3.2.1.147) was obtained from Sigma-Aldrich Co. UK.

5.2.2. Preparation of glucosinolate standard solutions

Solutions were prepared from glucoraphanin, gluconasturtiin gluconapin and mixture glucosinolates at different concentrations (25, 12.5, 6.25, 3.125, 1.56 Micromoles. L^{-1}) dissolved in distilled water. Myrosinase enzyme solution was prepared as (0.5 unit) by dissolving 100 enzyme units in 200 ml distilled water.

5.2.3. Seed bioassays

Seeds (250) of test weeds [Phalaris minor (Retz.) (canary grass), Convolvulus arvensis (L.) (field bindweed) and Sorghum halepanses (L.) (Johnsongrass)] were prepared as previously described (Chapter 2, section 2.2.2). Each solution (9 mls) was added to a separate Petri dish and assays were carried out in the presence and absence on myrosinase. One ml myrosinase was add to each Petri dish and distilled water was add as a control. All Petri dishes were placed in plant growth chambers (Sanyo MLR) at 25°C and 70% humidity and in continuous darkness. Treatments were arranged in a completely randomized design (CRD) with factorial

arrangements in five replications (4 pure glucosinolates solutions x 6 concentrations x 2 myrosinase enzyme).

5.2.4. Assessment

Germination, shoot and root length and fresh weight of seedlings were measured as as previously described (Chapter 2, section 2.2.3).

5.2.5. Statistical analysis

The experiment consisted of three factors (4 pure glucosinolates solutions x 6 concentrations x 2 myrosinase enzyme) with five replicates for each treatment arranged in a completely randomized design for each weed species.

Factorial ANOVA (pure glucosinolates x concentrations x myrosinase enzyme) was carried out to analyse the experiment results using GenStat® 15th Edition (VSN international, Hemel Hemstead, UK) for each weed species separately. A Duncan test was used to compare the differences between means of pure glucosinolates at different concentrations at level ($P < 0.05$) and is displayed in the (Appendix 6, 7 and 8). Regression analysis was also undertaken to determine the relationship between different characters.

5.3. Results

5.3.1. Effects of glucosinolates in the presence and absence of myrosinase on weed seed germination

Analysis of Variance (Table 5.1) showed that the different glucosinolates used at different concentrations with and without myrosinase significantly (P<0.001) inhibited seed germination (%) of *Phalaris minor* (Retz.), *Convolvulus arvensis* (L) and *Sorghum halepanses* (L.) as compared with control.

The germination of *P. minor* (Retz.), *C. arvensis* (L) and *S. halepense* (L.) was significantly (P<0001) inhibited in petri dishes when using all glucosinolates. Glucoraphanin was the most effective on seed germination (%) of all three weed species, and also gluconapin and gluconasturtiin were more inhibiting to seed germination (%) than the glucosinolate mixture (Figures. 5.1, 5.2 and 5.3 and Duncan test results in Appendix 6, 7 and 8).

Seed germination (%) of weed species at different concentrations of glucosinolates (Figures. 5.1, 5.2 and 5.3) indicated a significant suppression (P<0.001) in seed germination (%) under different glucosinolates concentration treatments. All concentrations of treatments significantly decreased the germination of all weed species. Higher concentrations of all glucosinolates used in the studies showed significantly maximum inhibition of seed germination (%) of weed species compared with other treatments. Also, myrosinase enzyme significantly suppressed seed germination (%) of weed species by increasing the effects of the glucosinolates (see Duncan test results in Appendix 6, 7 and 8).

Glucoraphanin and gluconapin at 25 µmol reduced the germination of *P. minor* (Retz.) up to 39.8% and 37.1% respectively and *S. halepense* (L) up to 35.2% and 28.4% respectively; however the gluconasturtiin at 25 µmol was more effective than the gluconapin when applied on *C. arvensis* (L) and the germination was decreased

up to 29.7% and by applying glucoraphanin at 25 µmol the germination reduced up to 33.1% (see Duncan test results in Appendix 6, 7 and 8).

Glucoraphanin and gluconapin with myrosinase enzyme were found be more effective on *P. minor* and *S. halepense* (L) germination compared with gluconasturtiin + myrosinase enzyme, however gluconasturtiin + myrosinase enzyme had more of an inhibitor effect on *C. arvensis* (L) compared with gluconapin+ myrosinase enzyme.

All glucosinolates at 25 µmol with myrosinase enzyme showed a significant inhibitory effect on seed germination (%) of all weed species (see Duncan test results in Appendix 6, 7 and 8).

5.3.2. Effects of glucosinolates in the presence and absence of myrosinase on shoot length (cm) of weed species

Data presented in Analysis of Variance (Table 5.2) reveals that there was a significant difference ($P \leq 0.001$) between glucosinolates and various concentrations with myrosinase enzyme and without myrosinase enzyme. Shoot length of *P. minor* (Retz.), *C. arvensis* (L) and *S. halepense* (L.) was highly significantly ($P < 0.001$) affected by glucosinolates, concentrations and myrosinase enzyme.

Glucoraphanin and glucosinolates mixture were found to be highly effective in inhibiting the growth of *P. minor* (Retz.), *C. arvensis* (L), while *S. halepense* (L.) shoot length was more effected by gluconapin and gluconasturtiin (Figures. 5.4, 5.5 and 5.6 and Duncan test results in Appendix 6, 7 and 8).

Shoot length of weed species were significantly reduced by various concentrations of glucosinolates. Furthermore, an increase in concentration demonstrated insignificant differences as compared with the control and lower concentration (Figures. 5.4, 5.5 and 5.6 and Duncan test results in Appendix 6, 7 and 8).

Glucoraphanin at 25 µmol was found to inhibit the shoot length of *P. minor* and *C. arvensis* by 30.7% and 28.6% respectively. Meanwhile, the gluconasturtiin at 25 µmol inhibits the shoot length of *S. halepense* by 25.8% compared with the control.

All glucosinolates with myrosinase enzyme significantly inhibited the shoot length of all three weed species compared with glucosinolates without myrosinase enzyme. Glucoraphanin with myrosinase enzyme inhibited the *P. minor* and *C. arvensis* by 23.3% and 25.2% respectively, while gluconapin with myrosinase enzyme suppressed shoot length of *S. halepense* by 23.8% (see Duncan test results in Appendix 6, 7 and 8).

Each concentration of each glucosinolate had a significant inhibitory effect on shoot length of all three weed species when the glucosinolates were applied with myrosinase enzyme (see Duncan test results in Appendix 6, 7 and 8).

5.3.3. Effects of glucosinolates in the presence and absence of myrosinase on root length (cm) of weed species

The results from Analysis of Variance (Table 5.3) revealed that glucosinolates used at various concentrations with and without myrosinase enzyme significantly (P<0.001) suppressed root length of *P. minor* (Retz.), *C. arvensis* (L) compared to the control. However, *S. halepense* (L.) root length was not significantly affected by glucosinolates.

The results showed that all glucosinolates significantly decreased *P. minor* and *C. arvensis* root length (Figures. 5.7, 5.8 and 5.9 and Duncan test results in Appendix 6, 7 and 8), glucosinolates mixture appeared the most effective in suppressing root length of *P. minor*, and the next most effective treatment when gluconapin applied. Glucoraphanin and ` gluconasturtiin significantly inhibited the *C. arvensis* root length. Various concentrations were used in the experiment; higher concentrations

25 µmol and 12.5 µmol of glucosinolates used in the studies demonstrated significantly higher inhibition of all three weed species root length as compared with other concentrations (Figures. 5.7, 5.8 and 5.9 and Duncan test results in Appendix 6, 7 and 8).

The highest reduction in root length of *P. minor* was reported in petri dishes treated with the glucosinolates mixture applied at 25 µmol and 12.5 µmol and the next best treatment was applying glucoraphanin at 25 µmol. Meanwhile, the greatest reduction in root length of *C. arvensis* was recorded when treated with gluconasturtiin applied at 25 µmol and also by glucoraphanin at 25 µmol. Applying gluconapin at 25 µmol was the most effective treatment on root length of *S. halepense*.

The results revealed that using different glucosinolates with myrosinase enzyme significantly inhibited the root length all three weed species. Also, the interaction between different glucosinolates with myrosinase enzyme with all concentrations significantly inhibited the root length all three weed species compared with the control (see Duncan test results in Appendix 6, 7 and 8).

5.3.4. Effects of glucosinolates in the presence and absence of myrosinase on shoot fresh weight (mg) of weed species

The results presented in Analysis of Variance (Table 5.4) revealed that there was a significant difference (P<0.01) among glucosinolates, various concentrations and myrosinase enzyme. Shoot fresh weight of *P. minor* (Retz.), *C. arvensis* (L) and *S. halepense* (L.) was highly significantly (P<0.01) affected by glucosinolates, concentrations and myrosinase enzyme.

All glucosinolates significantly inhibited all three weed species shoot fresh weight, while glucoraphanin appeared the most effective in suppressing shoot fresh weight

of *P. minor* followed by the glucosinolate mixture. Meanwhile, gluconasturtiin was the most effective on shoot fresh weight of *C. arvensis* and *S. halepense* (Figures 5.10, 5.11 and 5.12 and Duncan test results in Appendix 6, 7 and 8). Shoot fresh weight of all three weed species were significantly affected by all concentrations and shoot fresh weight reduction increased by increasing the concentrations, treatment of 25 µmol decreased shoot fresh weight *P. minor*, *C. arvensis* and *S. halepense* by up to 25%, 28.8% and 43.5% respectively (see Duncan test results in Appendix 6, 7 and 8).

All glucosinolate concentrations tested were found to be significant for shoot fresh weight of all three weed species. The highest reduction in shoot fresh weight of *P. minor* was recorded in treatment with glucoraphanin at concentrations of 25 µmol and 12.5 µmol, while the reduction in shoot fresh weight of *C. arvensis* occurred when treated by the glucosinolate mixture at concentration of 25 µmol, and also when treated by gluconapin at 25 µmol. Meanwhile, the highest reduction in fresh weight of *S. halepense* was recorded when treated with gluconasturtiin and gluconapin at concentration of 25 µmol.

Glucosinolates reduced the shoot fresh weight of all three weed species regardless of the addition of myrosinase. However, when glucosinolates were combined with myrosinase enzyme the were even more effective at reducing shoot fresh weight (Figures 5.10, 5.11 and 5.12 and Duncan test results in Appendix 6, 7 and 8). The glucosinolates mixture at 25 µmol and 12.5 µmol with myrosinase enzyme appeared the most effective in suppressing shoot fresh weight of *P. minor* compared with other treatments, the next best treatment was glucoraphanin at 25 µmol with myrosinase enzyme. Meanwhile, the highest reduction in shoot fresh weight of *C. arvensis* and *S. halepense* were recorded when treated by gluconapin at 25 µmol with myrosinase

enzyme, and also by gluconasturtiin at 25 µmol with myrosinase enzyme (see Duncan test results in Appendix 6, 7 and 8).

5.3.5. Effects of glucosinolates in the presence and absence of myrosinase on root fresh weight (mg) of weed species

Analysis of Variance (Table 5.5) revealed that different glucosinolates at various concentrations with and without myrosinase enzyme significantly (P<0.05) suppressed the root fresh weight of *P. minor* (Retz.) and *C. arvensis* (L). *Sorghum halepense* (L.) root fresh weight was also significantly (P<0.001) affected by all treatments individually and combination with myrosinase enzyme.

The results obtained on root fresh weight of weed species that were exposed to different glucosinolates indicated a significant suppression (P<0.001) under different concentrations of glucosinolate treatments (Figures 5.13, 5.14 and 5.15 and Duncan test results in Appendix 6, 7 and 8). All glucosinolate treatments significantly decreased the root fresh weight of all weed species. Gluconapin seemed to be the most effective in inhibiting root fresh weight of *P. minor*, followed by the glucosinolate mixture. The highest reduction in root fresh weight of *C. arvensis* was reported when treated with glucoraphanin followed by gluconasturtiin. However, gluconasturtiin was the most effective on *S. halepense* root fresh weight (Figures 5.13, 5.14 and 5.15 and Duncan test results in Appendix 6, 7 and 8). Higher concentration of all glucosinolates used in the studies showed maximum inhibition of root fresh weight of all three weed species compared with other treatments. (see Duncan test results in Appendix 6, 7 and 8).

The highest reduction in root fresh weight of *P. minor* was observed when treated with gluconapin at 25 and 12.5 µmol followed by gluconasturtiin at 25 µmol. Glucoraphanin and gluconasturtiin at the 25 µmol concentration caused the greatest

inhibition to *C. arvensis* and *S. halepense* root fresh weight (Figures 5.13, 5.14 and 5.15 and Duncan test results in Appendix 6, 7 and 8).

All glucosinolates with and without myrosinase enzyme significantly reduced the root fresh weight of all three weed species. However, glucosinolates with myrosinase enzyme were most effective on root fresh weight (Figures. 5.13, 5.14 and 5.15 and Duncan test results in Appendix 6, 7 and 8).

The results revealed that gluconapin at 25 µmol combined with myrosinase enzyme significantly inhibited the root fresh weight of *P. minor* and *C. arvensis* by 64.6% and 66.4% respectively. Glucoraphanin at 25 µmol concentration with myrosinase enzyme reduced the root fresh weight of *S. halepense* by 69%.

5.3.6. The relationships between different pure glucosinolates with myrosinase and seed germination (%)

Regression analysis showed positive linear relationships between different pure glucosinolates (glucoraphanin (i), gluconapin (ii), gluconasturtiin (iii) and mixed (GSLs) with myrosinase (iiii) and seed germination (%) of all three weed species after 14 days (Figures 5.16, 5.17 & 5.18).

The relationship was significant (P=0.05) for all pure glucosinolates (GSLs) concentration with seed germination % of *Phalaris minor, Convolvulus arvensis* & *Sorghum halepense*.

5.4. Discussion

A laboratory experiment was conducted to investigate the potential effect of using pure glucosinolates under various concentrations in the presence and absence of myrosinase enzyme on seed germination and seedling growth of three weed

species; *P. minor* (Retz.) (canary grass), *C. arvensis* (L.) (field bindweed) and *S. halepense* (L.) (Johnsongrass).

Seed germination and seedling growth of *P. minor* (Retz.), *C. arvensis* (L.) and *S. halepense* (L.) in laboratory bioassays were significantly supressed by applying pure glucosinolates at different concentrations and myrosinase enzyme. All pure glucosinolates treatments exhibited consistency in their ability to inhibit the germination (%) of all three weed species at different concentrations under laboratory conditions. Glucoraphanin applied with myrosinase enzyme was most effective and suppressed the weed species germination (%) regardless of the concentration used. The next best treatment was gluconapin at different concentrations with myrosinase enzyme especially with *P. minor* and *S. halepense*; however, gluconasturtiin at different concentrations with myrosinase enzyme was the next best affective treatment on *C. arvensis*.

Although the glucosinolates mixture applied with myrosinase had significant effects on germination (%) of all weed species, this treatment was the least effective on weed germination (Figures. 4.1, 4.2 and 4.3 and Duncan test results in Appendix 6, 7 and 8). The degree of suppression was mostly dependent on the concentration of the pure glucosinolates tested in this study, the inhibition of seed germination of all three species reduced largely by increasing the concentration glucoraphanin and gluconapin followed by gluconasturtiin.

The use of pure glucosinolates for weed management have not previously been reported. The closest reports to the results recorded were the experiments conducted to test the allelopathic potential of using *Brassicaceae* family members and using brassica crops as green manures. These findings are consistent with those of (Al-Khatib and Boydston, 1999), who found that members of the Brassicaceae family have a number of biologically active compounds including

glucosinolates and their hydrolysis products thiocyanates and isothiocyanates, which have the ability to reduce seed germination and plant growth. Also, Branca *et al*. (2002), reported that high levels of glucosinolates were found in several *Brassica spp*., which have ability to reduce the germination and seedling growth of plant species.

Moreover, similar results were obtained by Turk and Tawaha (2003), when they found that water extracts made using different plant parts from *B. nigra* (leaf, stem, flower and root) at different concentrations significantly affected germination, dry weight, shoot and root length. They also found that effectiveness increased significantly with increasing the water extract concentration of different parts of *B. nigra* (L.). According to Rice (1984), allelochemicals such as glucosinolates, may affect plant growth by affecting a number of physiological processes such as cell division and elongation, mineral and water uptake, stomatal opening and photosynthesis, membrane permeability, change in lipids, seed germinations, change organic acid metabolism, inhibition of enzymes and effect on xylem.

The probable cause of germination inhibition was glucosinolate producing toxins. Earlier research shows that examining extracts from glucosinolate producing plant species have an ability to inhibit germination (Brown and Morra, 1996; Al-Khatib *et al*., 1997; Norsworthy *et al*., 2007; Malik *et al*., 2008). Leblová-Svobodová and Koštir (1962) observed that the protein synthesis in seed germination and seedling emergence may be affected by chemical compounds formed from glucosinolate hydrolysis and this suppression may be due to of relatively large amounts of isothiocyanates absorbed compared to the seed mass, or that one of the first processes in seed germination was inhibited.

One of the secondary plant metabolites is glucosinolate which is found in *Brassica spp*., and myrosinase enzyme can hydrolysise the glucosinolates into toxic products like isothiocyanates that have the ability to control weed seeds (Brown and Morra, 1997; Al-Turki and Dick, 2003). Seed germination and seedling growth of numerous of weeds were inhibited by isothiocyanate (Brown and Morra, 1997; Al-Turki and Dick, 2003; Norsworthy *et al*., 2006; Bangarwa *et al*., 2010). Also, Jafariehyazdi and Javidfar (2011) suggested that the toxic effect of *Brassica spp*. may be caused by hydrolysis products of glucosinolates that occur in substantial amounts in the vegetative parts of *Brassica spp*.

The investigations showed that shoot length of all three weed species was significantly inhibited by all pure glucosinolate treatments at different concentrations and with myrosinase enzyme (Figures 4.4, 4.5 and 4.6 and Duncan test results in Appendix 6, 7 and 8). The average shoot length inhibition was increased by increasing the pure glucosinolate concentration. This might be due to the pure glucosinolates at different concentrations both with and without myrosinase enzyme, have a great potential to minimize shoot length and it probably affects cell division and elongation by interacting with production or transport of plant growth regulators such as, cytokinins and auxins that stimulate cell division and cell elongation (Rice,1984).

Similar observations were made with root length of all three weed species treated by all pure glucosinolate treatments at different concentrations and with myrosinase enzyme (Figures. 4.7, 4.8 and 4.9 and Duncan test results in Appendix 6, 7 and 8). However, the root length was affected more than that of the shoot length. This might be because of the direct contact of root with the inhibitory chemicals pure glucosinolates (Quasem, 1995). The results show (Figures 4.10, 4.11 and 4.12 and Duncan test results in Appendix 6, 7 and 8), that shoot and root fresh weight were

significantly decreased by all pure glucosinolate treatments at different concentrations with and without myrosinase enzyme. The reduction in shoot and root fresh weight may be attributed to the inhibition in shoot and root length. This finding concurs with results observed by Obaid and Qasem (2005).

In the present study, the results clearly confirm that all glucosinolates at different concentrations without enzymes significantly affect all three weed species. Seed germination (%), shoot and root length, shoot and root fresh weight were inhibited significantly; however, these reductions in all parameters were less compared with all treatments with myrosinase enzyme. It might be due to potential toxicity levels caused by pure glucosinolates and this may lead to affecting the water uptake by increasing the pure glucosinolates concentrations. Jafariehyazdi and Javidfar (2011) suggested that that water uptake was reduced by increasing the concentration of aqueous extracts from *B. napus*, *B. rapa* and *B. juncea*.

These results are in contrast to previous results reported by several researchers including; (Leblová-Svobodová and Koštir, 1962; Brown and Morra, 1996; Brown and Morra, 1997; Al-Khatib *et al*., 1997; Rask *et al*., 2000; Norsworthy *et al*., 2007; Al-Turki and Dick, 2003; Wittstock *et al*,. 2004; Song *et al*., 2005; Bennett *et al*., 2006; Norsworthy *et al*., 2006; Malik *et al*., 2008; Bangarwa *et al*., 2010). Their research suggested that isothiocyanates are primarily released when brassica plant residues are hydrolysed, and the effect of undamaged plant tissue which contains glucosinolates is very little on the other organism. However, the results of using pure glucosinolates with and without myrosinase in the laboratory were not clear in comparison with those previously reported by the several researchers because they may have used a different experimental methodology under different conditions.

5.5. Conclusions

The results of this experiment have demonstrated that all pure glucosinolates with and without myrosinase enzyme significantly reduced germination %, shoot and root length, shoot and root fresh weight for all three weed species. The greatest effect in all parameters of three weed species was obtained with using pure glucosinolates with myrosinase enzyme compared with the pure glucosinolates without myrosinase enzyme. Glucoraphanin at 25 µmol the most showed the greatest inhibitory effect on *C. arvensis* germination, shoot and root length and root weight, but shoot weight was also reduced by gluconasturtiin at 25 µmol. Glucoraphanin at 25 µmol was the effective treatment on *P. minor* germination %, shoot length and shoot fresh weight, however, root length and weight effected by gluconapin at 25 µmol. Also, gluconapin at 25 µmol was the most effective treatment on *S. halepense* germination, shoot and root length, but shoot and root fresh weight were more effected by gluconasturtiin at 25 µmol.

Table 5.1. Analysis of variance relating to the effect of different glucosinolates with various concentrations and myrosinase enzyme on seed germination % of weed species

Source of variation	d.f.	Mean squares		
		Germination (%)		
		Phalaris minor	*Convolvulus arvensis*	*Sorghum halepense*
Glucosinolates	3	955.8**	308.2**	595.1**
Concentrations	5	4296.9**	2894**	2123.4**
Myrosinase enzyme	1	18375**	5415**	6355.1**
Glucosinolates x Concentrations	3	118.8**	40.4**	66.4**
Glucosinolates x Myrosinase enzyme	5	831.9**	202.8**	461.3**
Concentrations x Myrosinase enzyme	15	1508.8**	477.3**	768.4**
Glucosinolates x Concentrations x Myrosinase enzyme	15	130.4**	42.4**	77.8**
Residual	192	9.53	19.38	5.365
Total	239			
CV%		4.1	6.1	3.4
SEM		3.087	4.402	2.316

**Significant at 1% probability level, *Significant at 5% probability level, NS = Non-significant

Table 5.2. Analysis of variance relating to the effect of different glucosinolates with various concentrations and myrosinase enzyme on shoot length (cm) of weed species

Source of variation	d.f.	Mean squares		
		Shoot length		
		Phalaris minor	*Convolvulus arvensis*	*Sorghum halepense*
Glucosinolates	3	1.81**	3.89**	20.42**
Concentrations	5	17.03**	63.97**	73.89**
Myrosinase enzyme	1	32.05**	434.16**	310.08**
Glucosinolates x Concentrations	3	0.67**	0.25^{NS}	1.65**
Glucosinolates x Myrosinase enzyme	5	6.62**	0.84**	20.07**
Concentrations x Myrosinase enzyme	15	3.46**	16.69**	23.28**
Glucosinolates x Concentrations x Myrosinase enzyme	15	0.69**	0.27**	1.49**
Residual	192	0.042	0.231	0.155
Total	239			
CV%		3.2	4.6	2.7
SEM		0.2049	0.4808	0.3941

**Significant at 1% probability level, *Significant at 5% probability level, NS = Non-significant

Table 5.3. Analysis of variance relating to the effect of different glucosinolates with various concentrations and myrosinase enzyme on root length (cm) of weed species

Source of variation	d.f.	Mean squares		
		Root length		
		Phalaris minor	*Convolvulus arvensis*	*Sorghum halepense*
Glucosinolates	3	0.57***	20.56***	0.2 ^{NS}
Concentrations	5	11.25***	70.78***	186.77***
Myrosinase enzyme	1	34.73***	910.26***	4177.5***
Glucosinolates x Concentrations	3	0.17***	1.57***	0.057^{NS}
Glucosinolates x Myrosinase enzyme	5	3.25***	6.83***	1.29***
Concentrations x Myrosinase enzyme	15	1.31***	49.11***	153.46***
Glucosinolates x Concentrations x Myrosinase enzyme	15	0.14***	1.49***	0.07 ^{NS}
Residual	192	0.048	0.120	0.142
Total	239			
CV%		5.4	4.8	4.2
SEM		0.2195	0.3467	0.3772

***Significant at 1% probability level, *Significant at 5% probability level, NS = Non-significant

Table 5.4. Analysis of variance relating to the effect of different glucosinolates with various concentrations and myrosinase enzyme on shoot fresh weight (mg) of weed species

Source of variation	d.f.	Mean squares		
		Shoot fresh weight		
		Phalaris minor	*Convolvulus arvensis*	*Sorghum halepense*
Glucosinolates	3	7.733**	217.33 **	843.60**
Concentrations	5	24.416**	6763.5**	4383.2**
Myrosinase enzyme	1	75.264**	87439.8**	37813.2**
Glucosinolates x Concentrations	3	0.231**	121.47**	26.25**
Glucosinolates x Myrosinase enzyme	5	1.677**	1060.9**	154.73**
Concentrations x Myrosinase enzyme	15	4.383**	3626.5**	1322.9**
Glucosinolates x Concentrations x Myrosinase enzyme	15	0.466**	94.51**	24.76**
Residual	192	0.0725	26.38	10.91
Total	239			
CV%		3.7	5	6.8
SEM		0.2693	5.136	3.303

**Significant at 1% probability level, *Significant at 5% probability level, NS = Non-significant

Table 5.5. Analysis of variance relating to the effect of different glucosinolates with various concentrations and myrosinase enzyme on root fresh weight (mg) of weed species

Source of variation	d.f.	Mean squares		
		Root fresh weight		
		Phalaris minor	*Convolvulus arvensis*	*Sorghum halepense*
Glucosinolates	3	1.00***	177.92 ***	1.54***
Concentrations	5	17.63***	370.79***	18.78***
Myrosinase enzyme	1	66.04***	3271.55***	235.18***
Glucosinolates x Concentrations	3	0.15**	5.24***	0.095^{NS}
Glucosinolates x Myrosinase enzyme	5	2.37***	88.27***	0.103^{NS}
Concentrations x Myrosinase enzyme	15	1.43***	167.95***	9.11***
Glucosinolates x Concentrations x Myrosinase enzyme	15	0.135**	5.14**	0.07^{NS}
Residual	192	0.065	2.02	0.07
Total	239			
CV%		7.8	7.6	6.9
SEM		0.256	1.4206	0.265

*** Highly significant at (P<0.001) probability level, **Significant at 1% probability level, NS = Non-significant

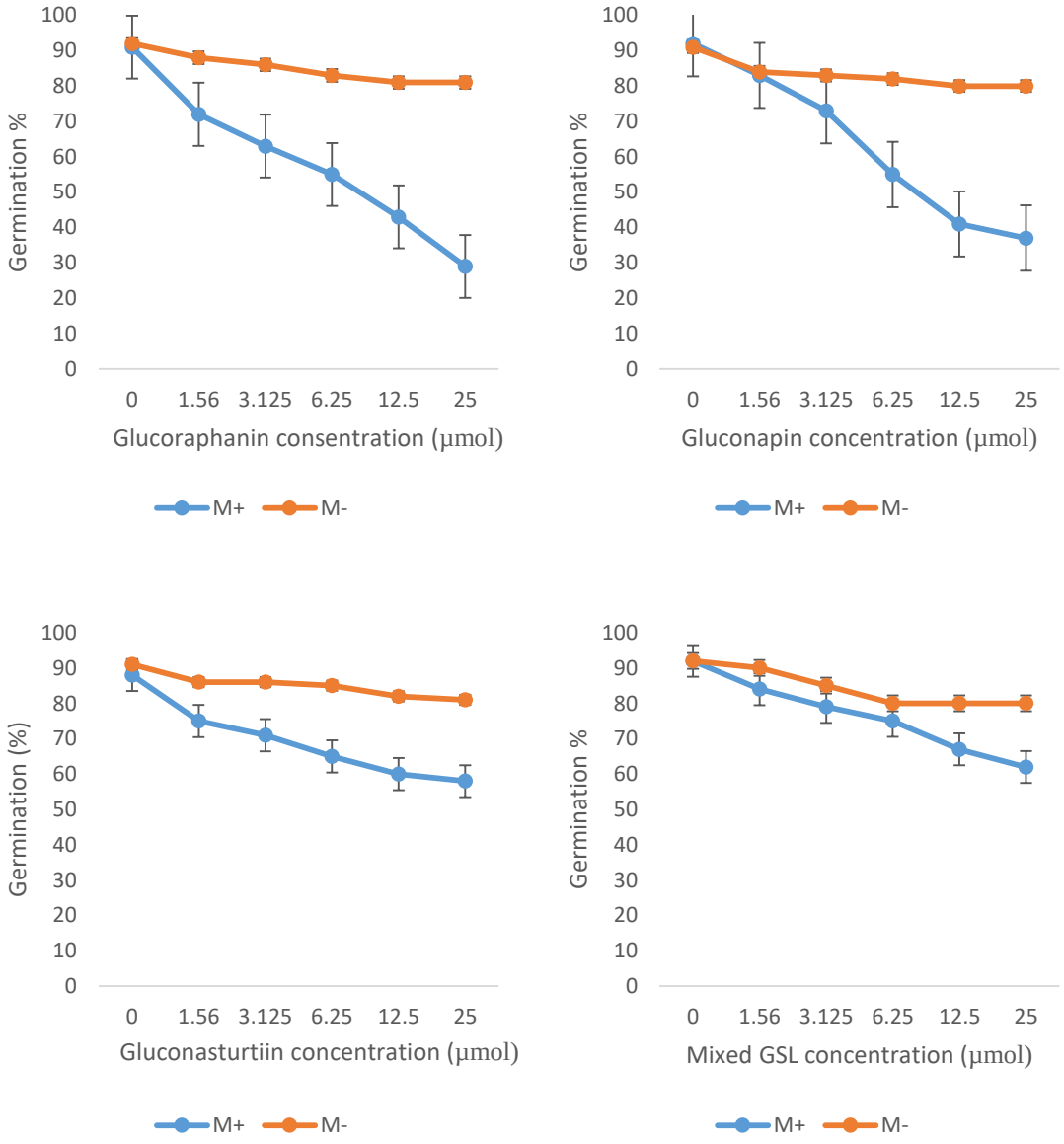

Figure 5.1. Effect of various concentrations of glucosinolates with (M+) myrosinase enzyme and without myrosinase (-M) on the seed germination (%) of *Phalaris minor* 14 days after sowing. Error bars represent the standard error of the mean. n = 240 for each treatment.

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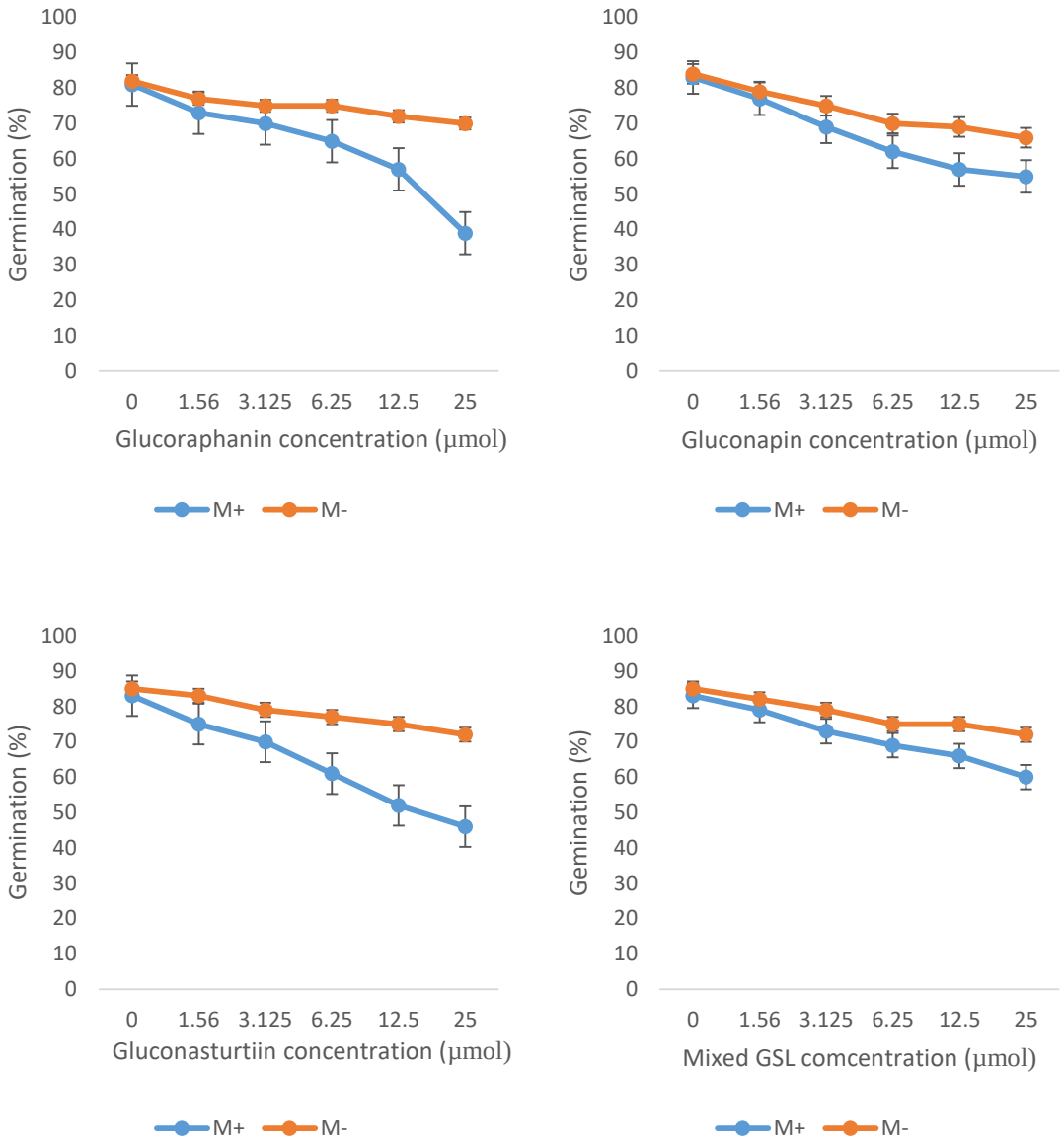

Figure 5.2. Effect of various concentrations of glucosinolates with (M+) myrosinase enzyme and without myrosinase (-M) on the seed germination (%) of *Convolvulus arvensis* 14 days after sowing. Error bars represent the standard error of the mean. n = 240 for each treatment.

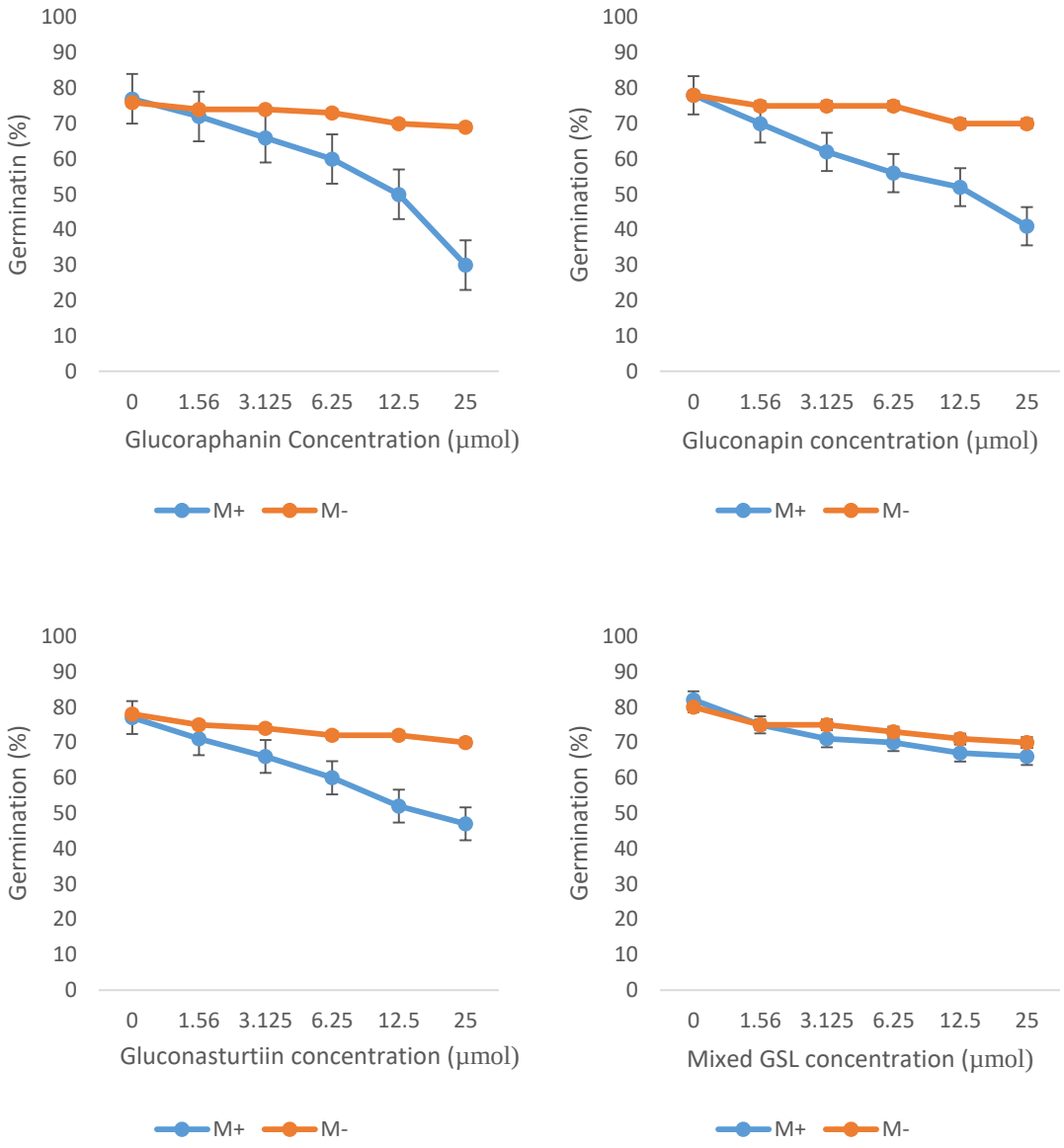

Figure 5.3. Effect of various concentrations of glucosinolates with (M+) myrosinase enzyme and without myrosinase (-M) on the seed germination (%) of *Sorghum halepense* 14 days after sowing. Error bars represent the standard error of the mean. n = 240 for each treatment.

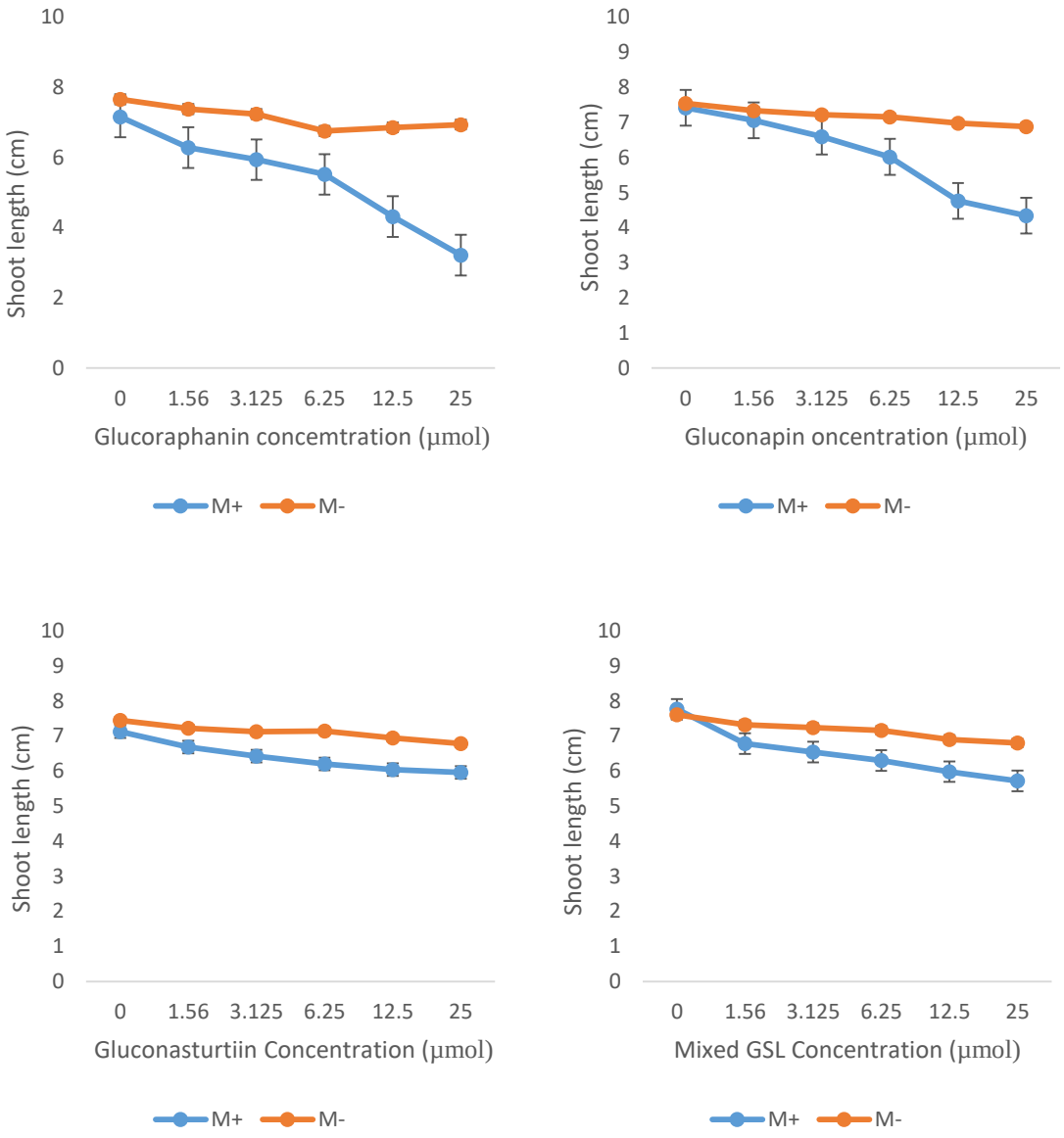

Figure 5.4. Effect of various concentrations of glucosinolates with (M+) myrosinase enzyme and without myrosinase (-M) on the shoot length (cm) of *Phalaris minor* 14 days after sowing. Error bars represent the standard error of the mean. n = 240 for each treatment.

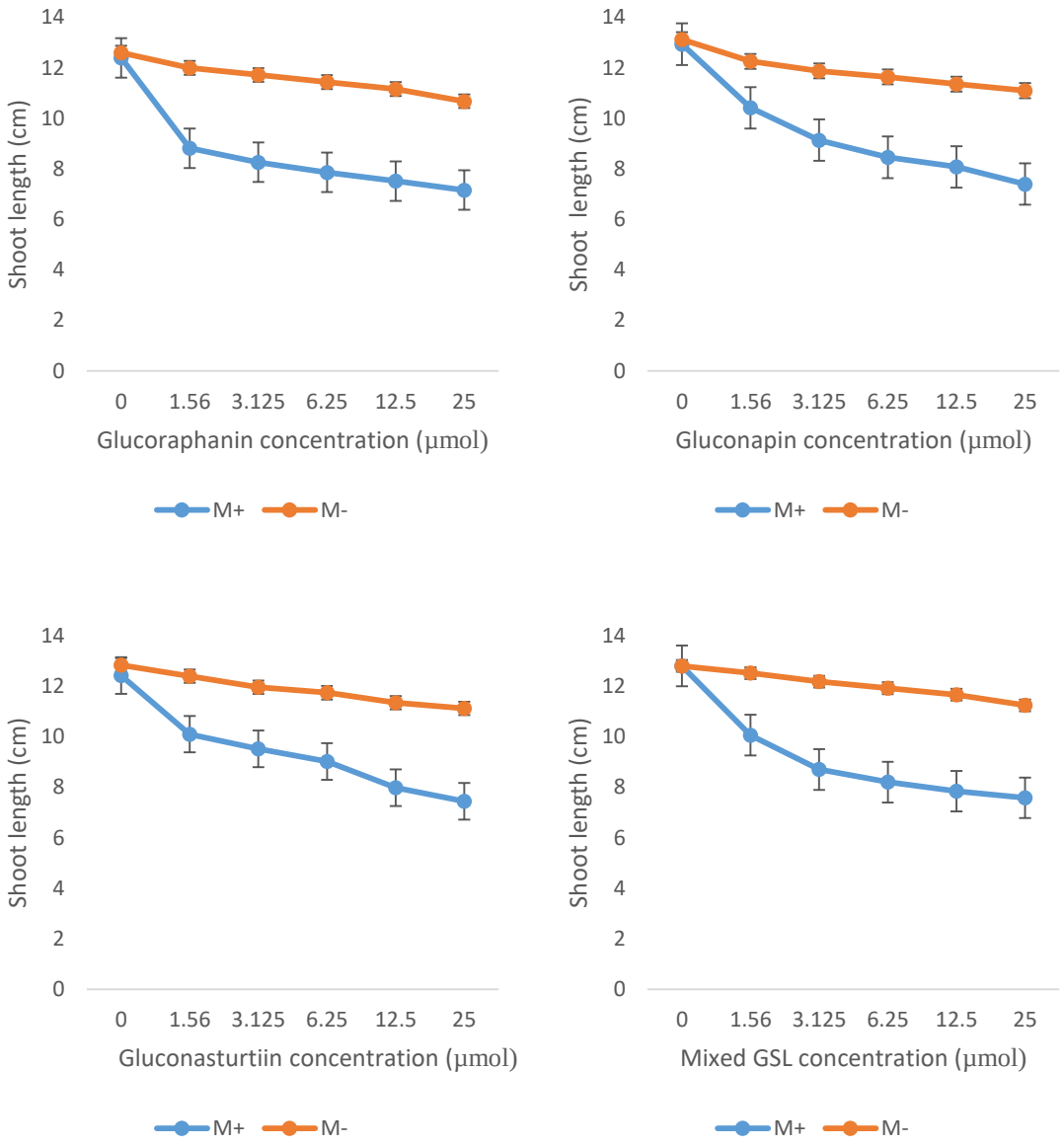

Figure 5.5. Effect of various concentrations of glucosinolates with (M+) myrosinase enzyme and without myrosinase (-M) on the shoot length (cm) of *Convolvulus arvensis* 14 days after sowing. Error bars represent the standard error of the mean. n = 240 for each treatment.

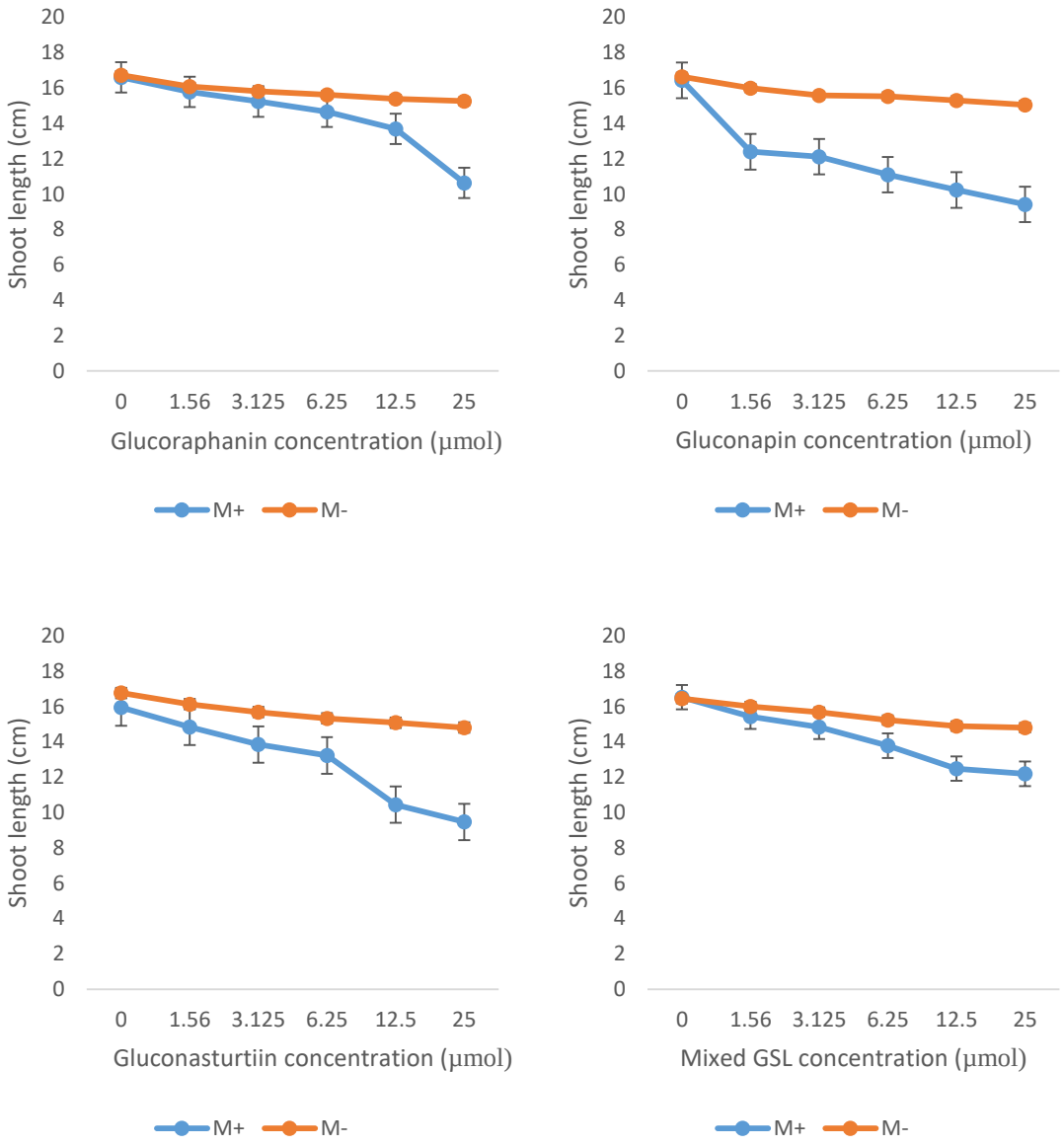

Figure 5.6. Effect of various concentrations of glucosinolates with (M+) myrosinase enzyme and without myrosinase (-M) on the shoot length (cm) of *Sorghum halepense* 14 days after sowing. Error bars represent the standard error of the mean. n = 240 for each treatment.

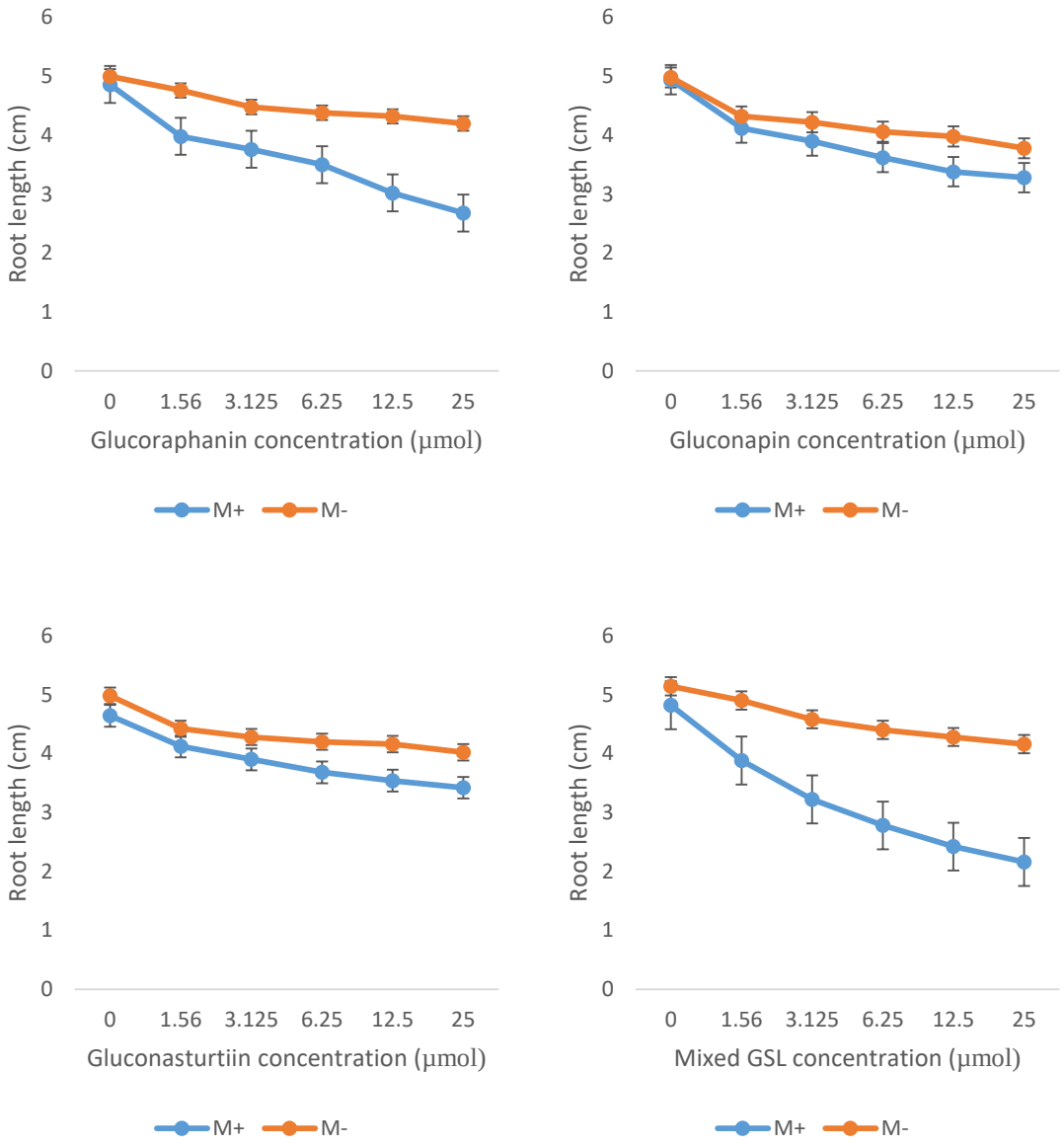

Figure 5.7. Effect of various concentrations of glucosinolates with (M+) myrosinase enzyme and without myrosinase (-M) on the root length (cm) of *Phalaris minor* 14 days after sowing. Error bars represent the standard error of the mean. n = 240 for each treatment.

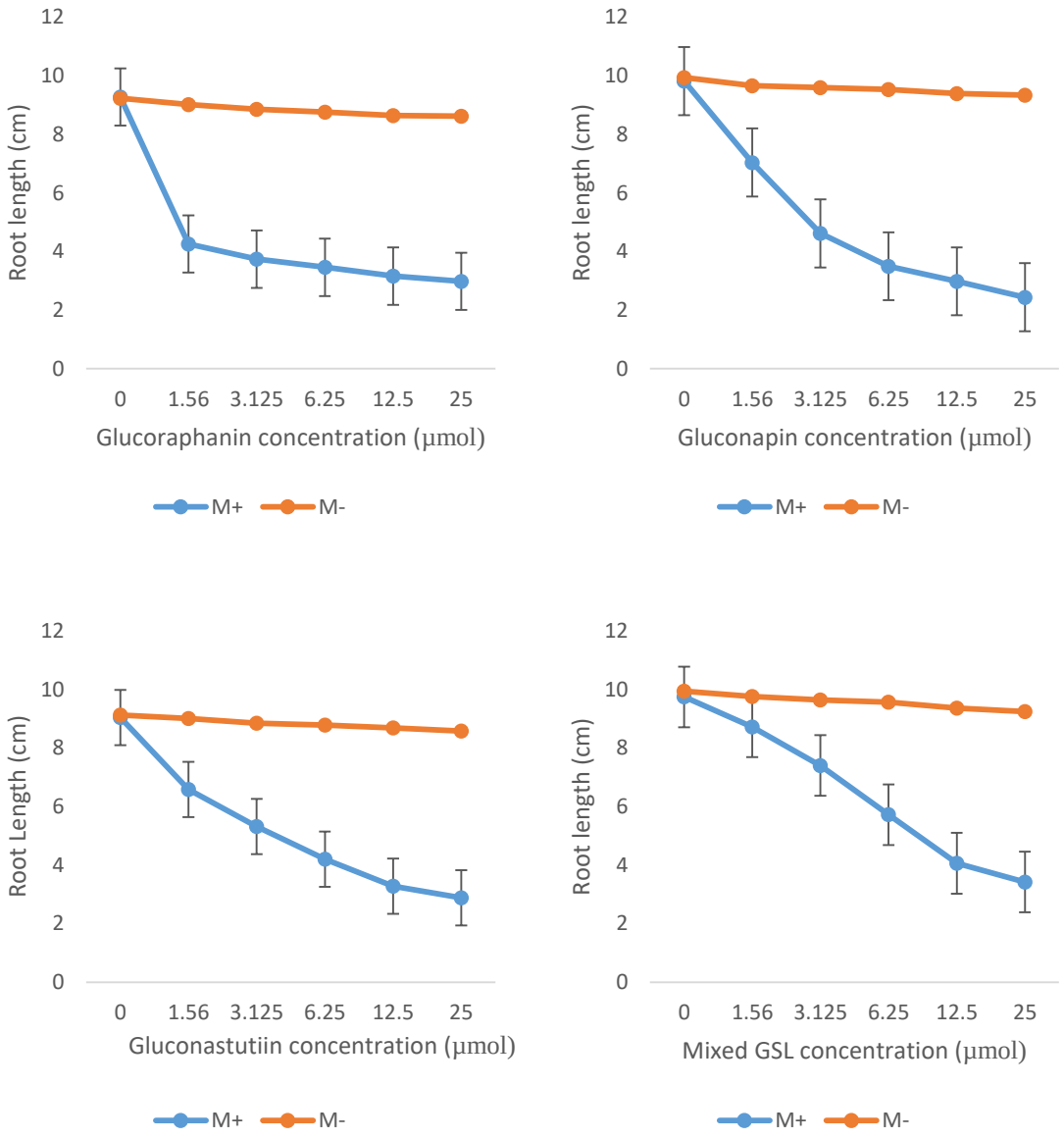

Figure 5.8. Effect of various concentrations of glucosinolates with (M+) myrosinase enzyme and without myrosinase (-M) on the root length (cm) of *Convolvulus arvensis* 14 days after sowing. Error bars represent the standard error of the mean. n = 240 for each treatment.

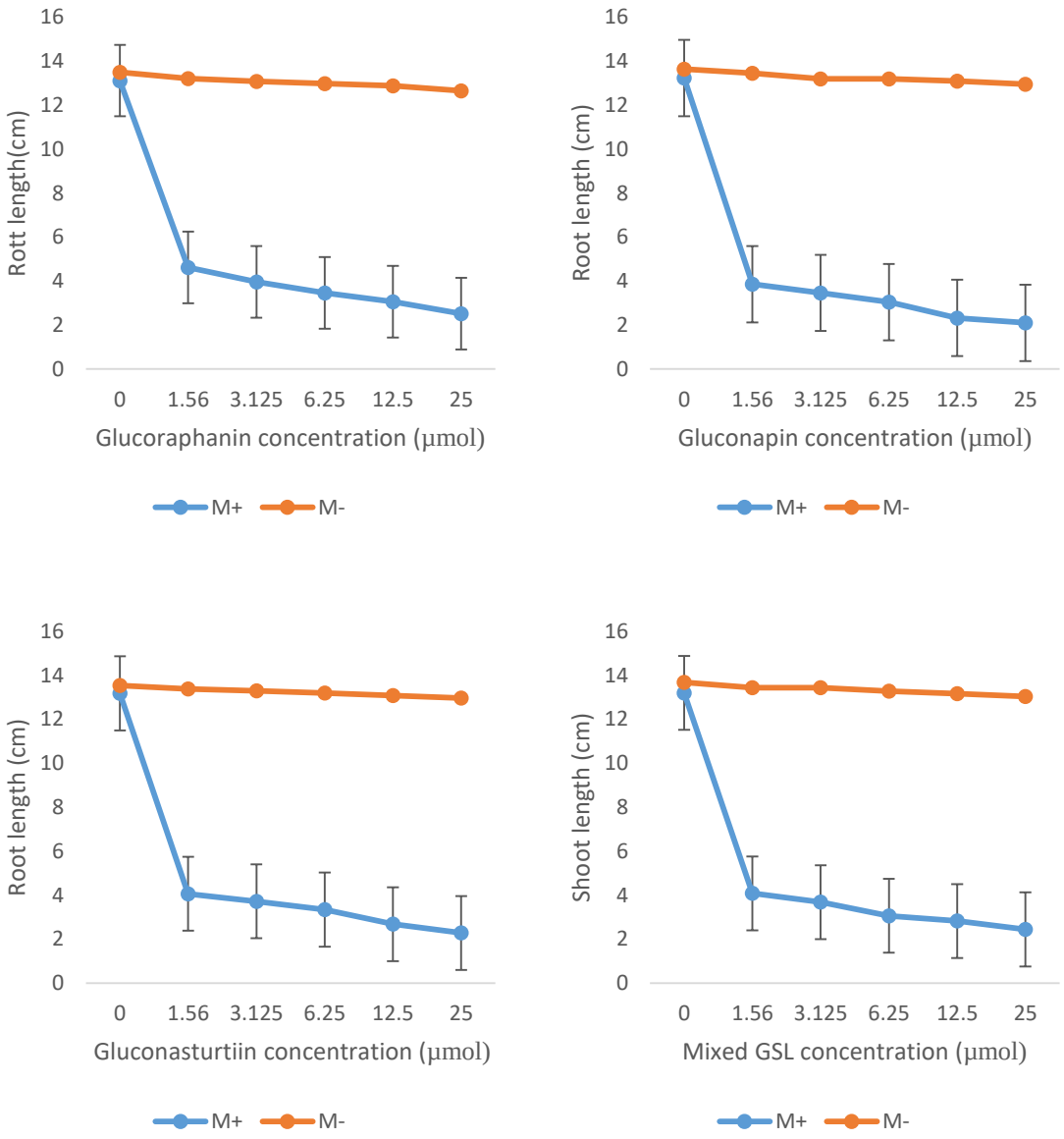

Figure 5.9. Effect of various concentrations of glucosinolates with (M+) myrosinase enzyme and without myrosinase (-M) on the root length (cm) of *Sorghum halepense* 14 days after sowing. Error bars represent the standard error of the mean. n = 240 for each treatment.

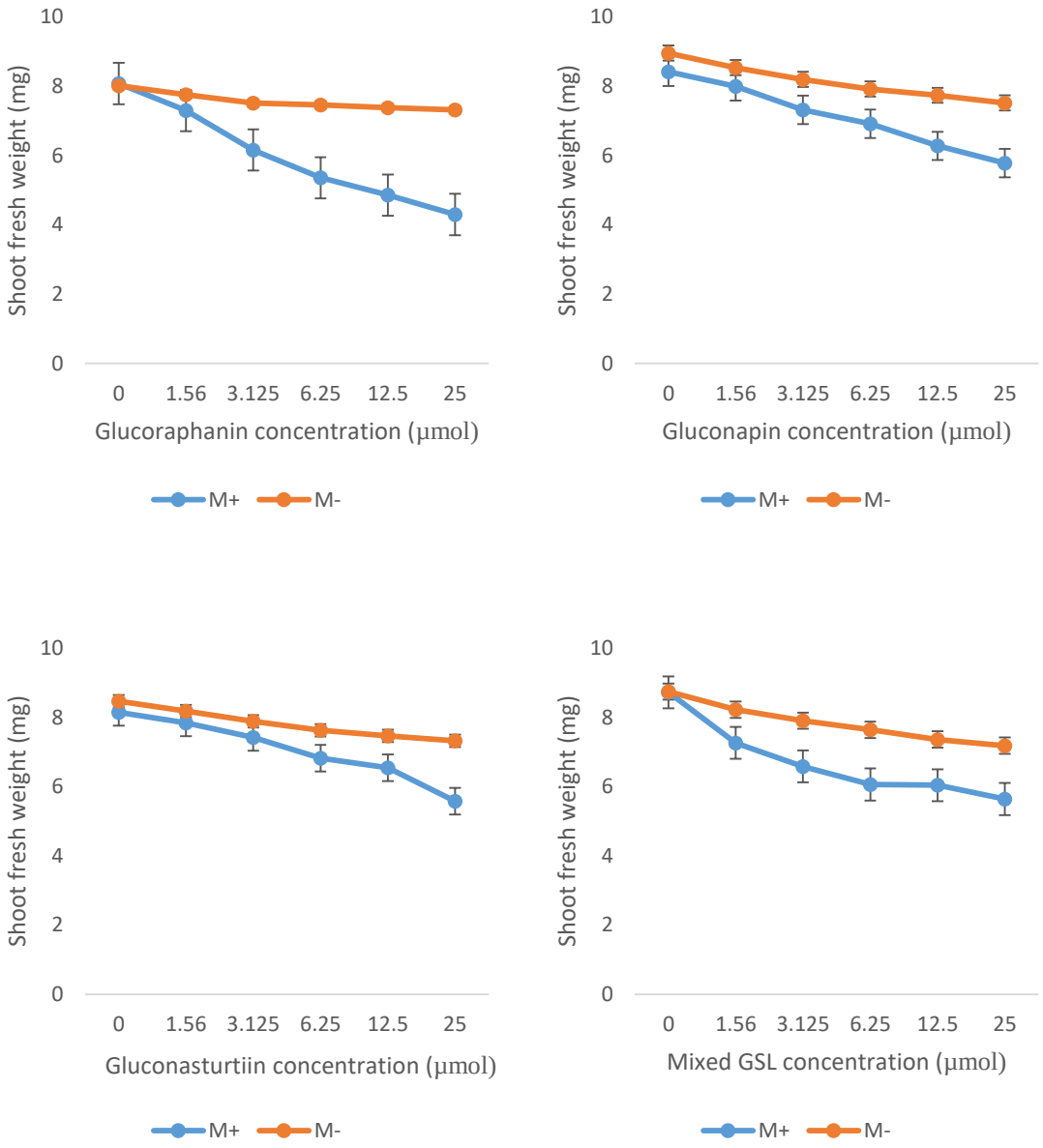

Figure 5.10. Effect of various concentrations of glucosinolates with (M+) myrosinase enzyme and without myrosinase (-M) on the shoot fresh weight (mg) of *Phalaris minor* 14 days after sowing. Error bars represent the standard error of the mean. n = 240 for each treatment.

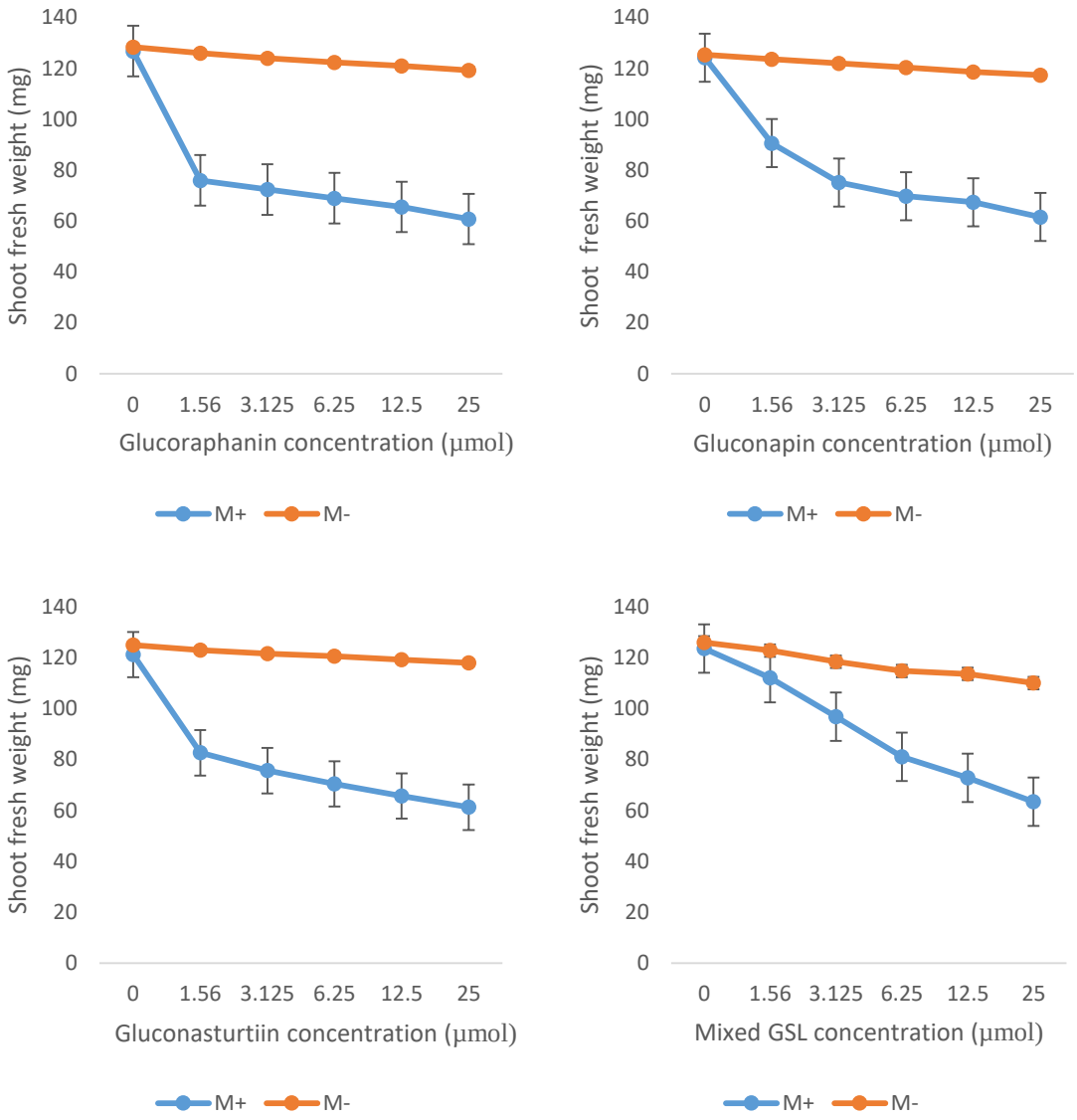

Figure 5.11. Effect of various concentrations of glucosinolates with (M+) myrosinase enzyme and without myrosinase (-M) on the shoot fresh weight (mg) of *Convolvulus arvensis* 14 days after sowing. Error bars represent the standard error of the mean. n = 240 for each treatment.

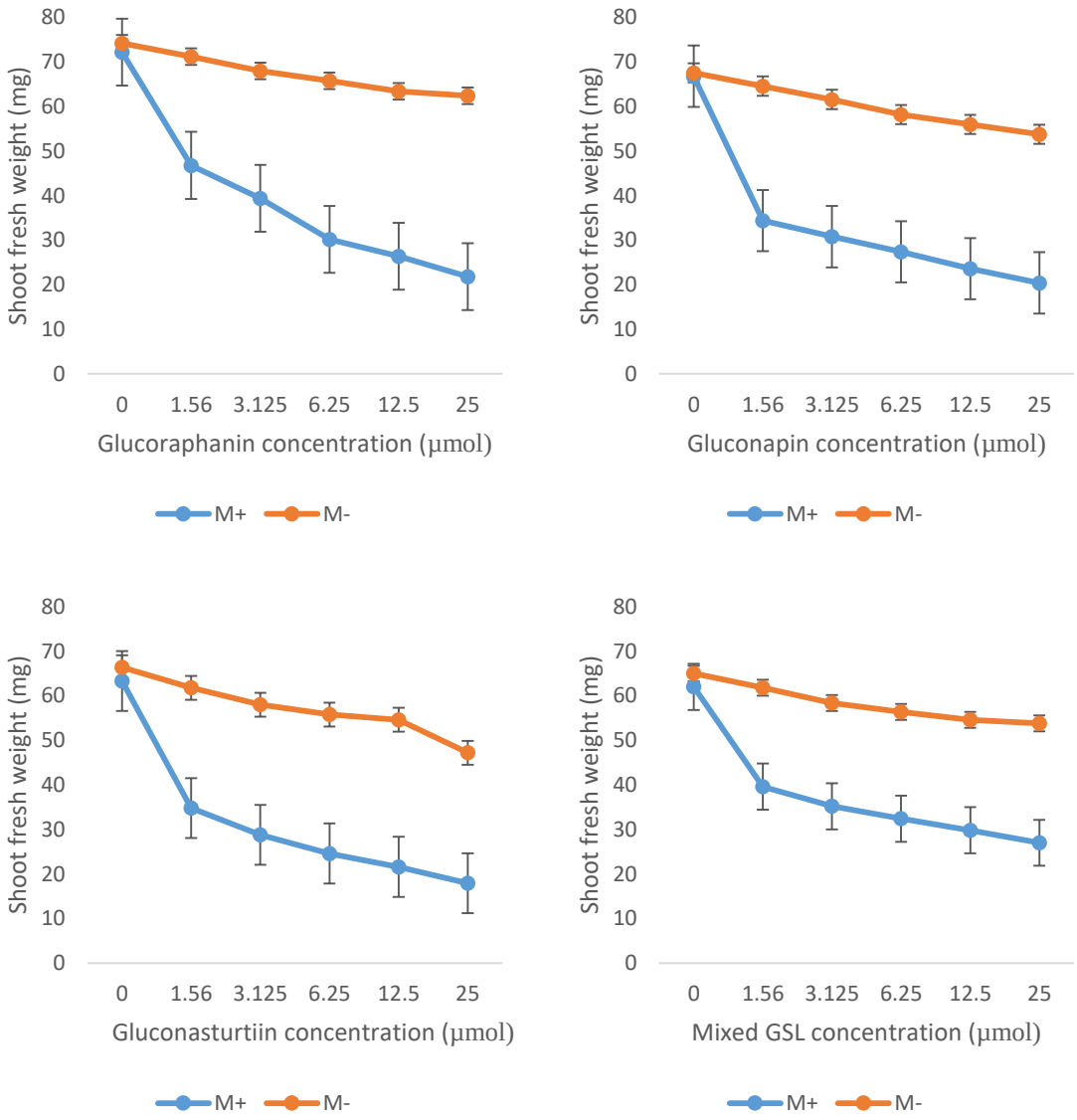

Figure 5.12. Effect of various concentrations of glucosinolates with (M+) myrosinase enzyme and without myrosinase (-M) on the shoot fresh weight (mg) of *Sorghum halepense* 14 days after sowing. Error bars represent the standard error of the mean. n = 240 for each treatment.

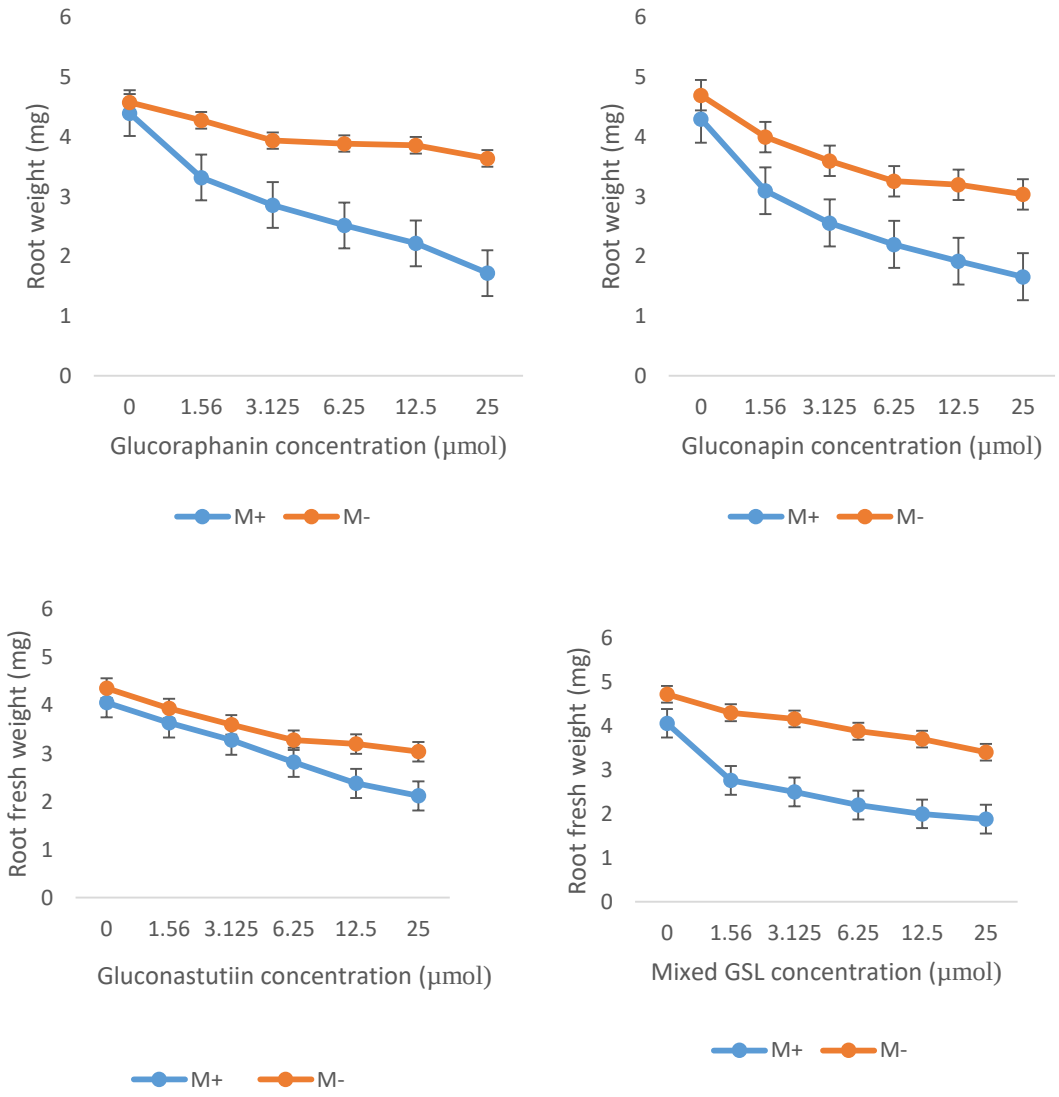

Figure 5.13. Effect of various concentrations of glucosinolates with (M+) myrosinase enzyme and without myrosinase (-M) on the root fresh weight (mg) of *Phalaris minor* 14 days after sowing. Error bars represent the standard error of the mean. n = 240 for each treatment.

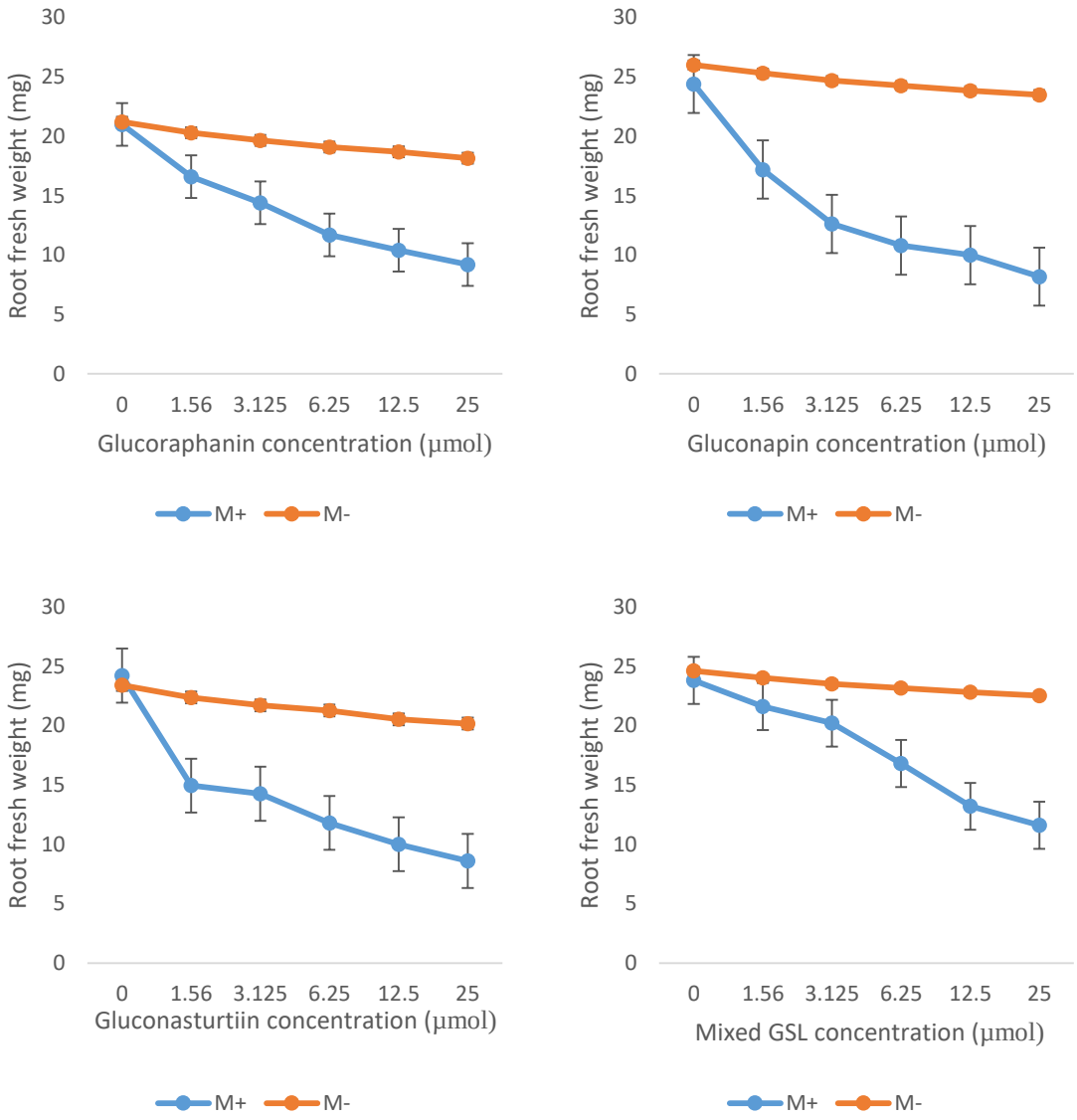

Figure 5.14. Effect of various concentrations of glucosinolates with (M+) myrosinase enzyme and without myrosinase (-M) on the root fresh weight (mg) of *Convolvulus arvensis* 14 days after sowing. Error bars represent the standard error of the mean. n = 240 for each treatment.

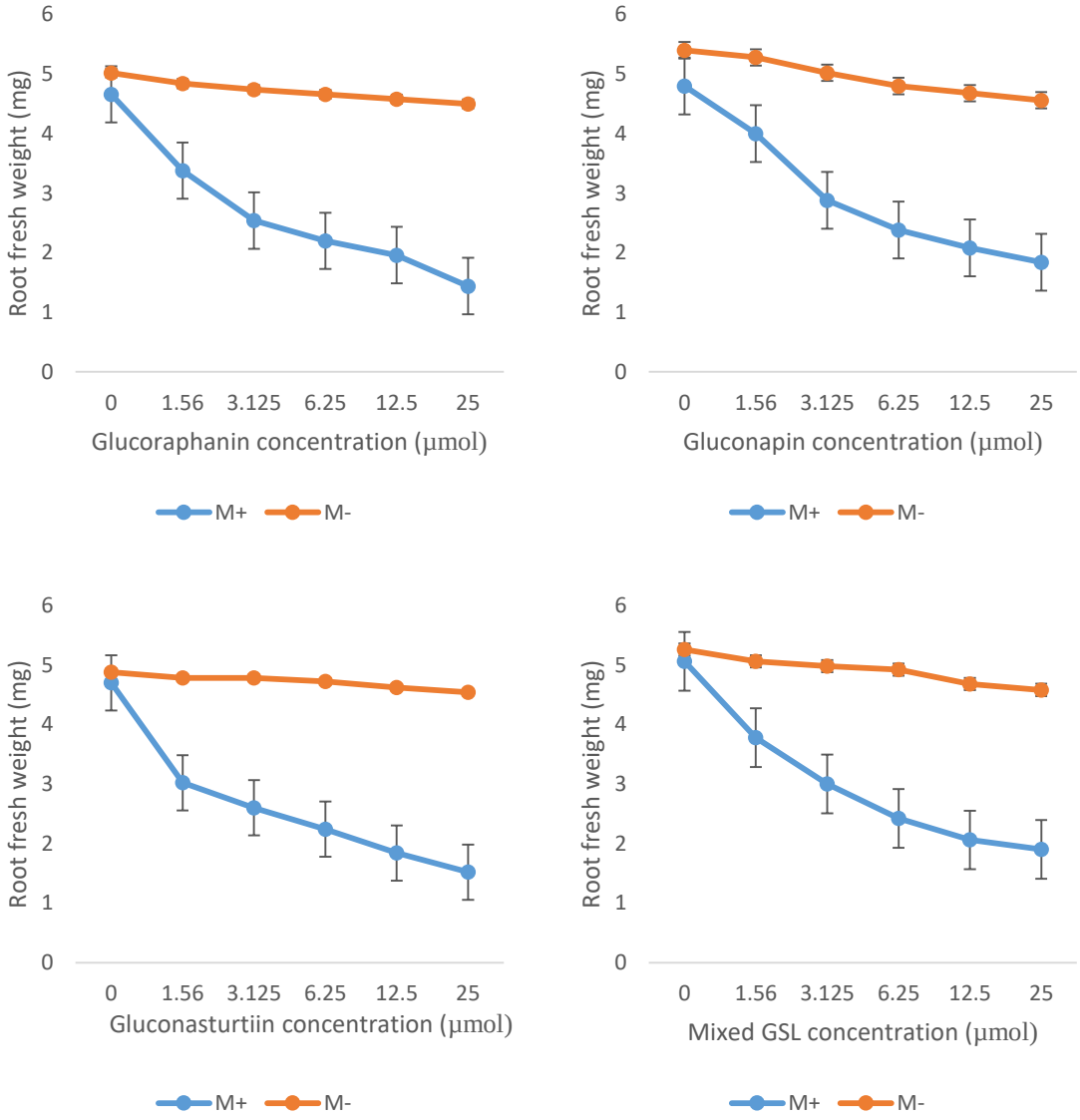

Figure 5.15. Effect of various concentrations of glucosinolates with (M+) myrosinase enzyme and without myrosinase (-M) on the root fresh weight (mg) of *Sorghum halepense* 14 days after sowing. Error bars represent the standard error of the mean. n = 240 for each treatment.

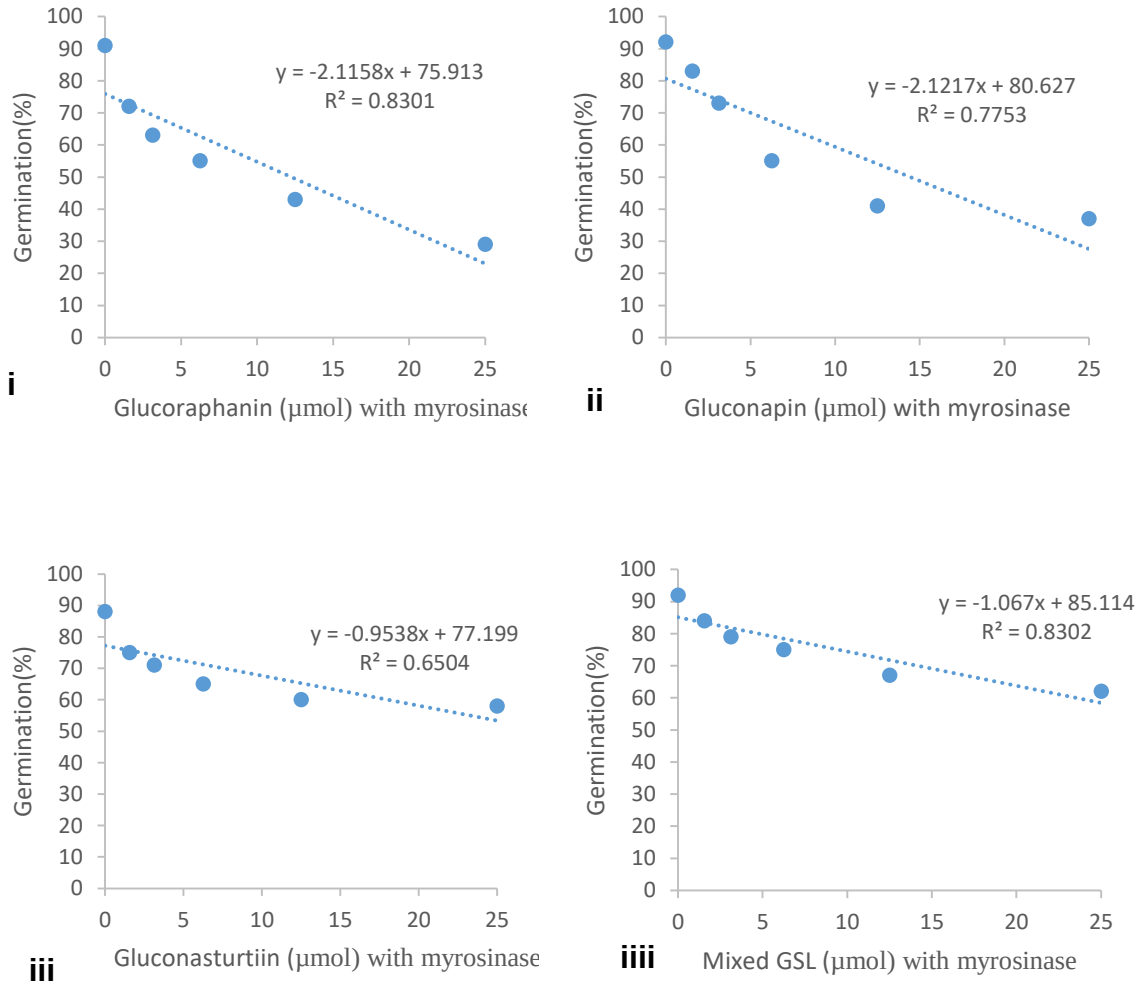

Figure 5.16. Relationships between seed germination (%) of *Phalaris minor* and pure glucosinolates (GSLs) concentration with myrosinase after 14 days.

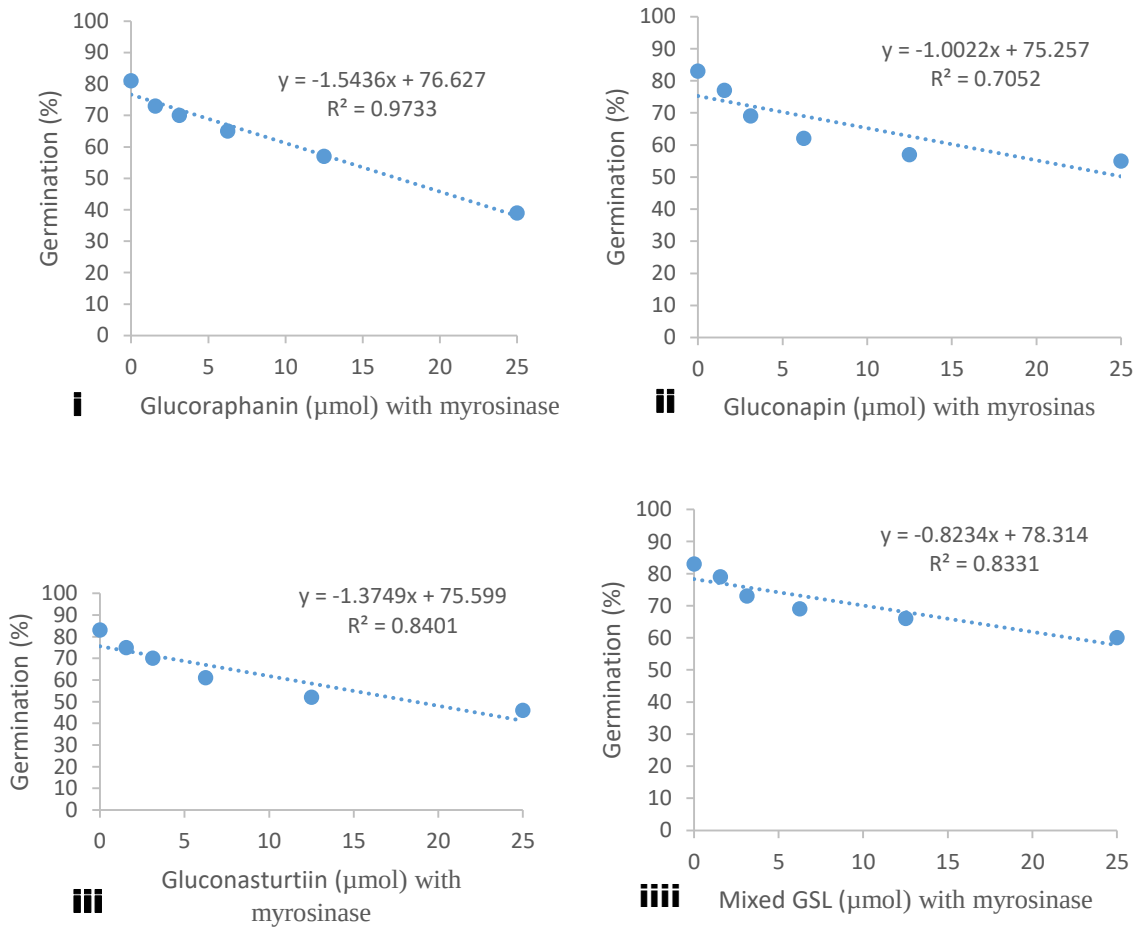

Figure 5.17. Relationships between seed germination (%) of *Convolvulus arvensis* and pure glucosinolates (GSLs) concentration with myrosinase after 14 days.

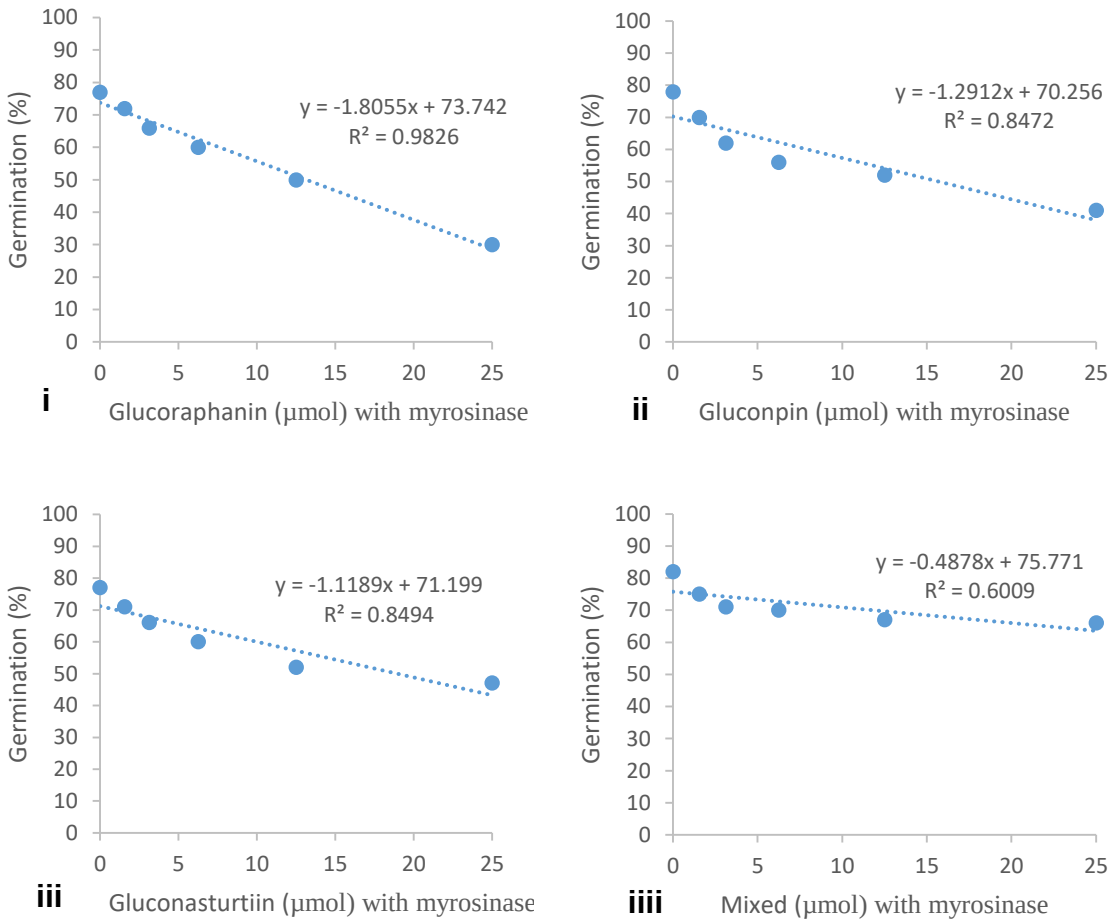

Figure 5.18. Relationships between seed germination (%) of *Sorghum halepense* and pure glucosinolates (GSLs) concentration with myrosinase after 14 days.

Chapter 6

6. Influence of different levels of water stress on allelopathic impact of *Brassica napus* L. tissues collected at different development stages on the suppression of weed species

6.1 Introduction

As the results from chapters 2, 3 and 4 showed, it is clear that *Brassica napus* water extract from different plant parts had a significant inhibitor effect on weed species germination and their seedling growth. This effect was documented not only in response to certain concentrations but under different concentrations and at different plant development stages. In addition, the weed species were significantly affected when treated by pure glucosinolates with myrosinase as the results shown in chapter 5. These effects may have been due to secondary plant metabolites mainly glucosinolates in *B. napus* water extract.

It is well documented as presented in Chapter 1 that glucosinolate hydrolysis by myrosinase results in the production of biocidal products such as isothiocyanates, which have the ability to inhibit weeds seed germination and seedling growth (Brown and Morra, 1997; Al-Turki and Dick, 2003; Norsworthy *et al*., 2006; Bangarwa *et al*., 2010). Suppression of weeds is increased by using brassica tissues with higher glucosinolate content (Giamoustaris and Mithen, 1995; Mithen, 2001; Al-Turki and Dick, 2003; Tawaha and Turk, 2003). Therefore, several studies have been focused on increasing the levels of glucosinolate in various brassica tissues by differing ways such as sowing at different times (seasonal effects), under different temperatures or water stress (Booth *et al*., 1991; Bennett *et al*., 1995; Bellostas *et al*., 2004; Justen *et al*., 2011; Bhushan *et al*., 2013). One of the major abiotic stresses which may affect plant physiology and, subsequently plant development is water stress (Zhu, 2001; Munns and Tester, 2008). Glucosinolate accumulation in brassica species *Nasturtium officinale* Engelen-Eigles (2006), *Brassica oleracea* (Champolivier and Merrien 1996; Paschold et al., 2000; Radovich *et al*., 2005), *Brassica napus* (Jensen *et al*., 1996), Brassica *rapa* ssp. (Zhang et al., 2008) and *Brassica carinata* (Schreiner *et al*., 2009), increased under water stress, and also may reduce plant

growth parameters following an increase of secondary metabolites at the expense of primary metabolism (Jones and Hartley, 1999).

6.2. Aims

i. The purpose of this study is to evaluate the effect of water stress on levels of glucosinolates and myrosinase activity from different parts of *Brassica napus*

ii. To determine the effect of *Brassica napus* water extracts from different parts at different water stress levels on weed species inhibition.

6.3. Materials and Methods

6.3.1. Experimental set-up

A Poly-tunnel experiment was conducted during March 2014 at Harper Adams University, Edgmond, Newport, Shropshire, England, UK. Oilseed rape (*Brassica napus L.*) cv. PR46W21 plants were grown in plastic pots of 19 cm (up diameter). The pots were filled with 2950 grams of (John Innes No. 2, sterilised loam based compost, Norwich, UK). Five seeds were planted in each pot and after two weeks plants were thinned to 2 plants per pot prior to the start of the treatment.

Plants were subjected to four different levels of water stress (soil moisture stress) treatments [WS1= 30% of field capacity (F.C.), WS2= 50% of F.C., WS3= 70 % of F.C. and WS4= 100% of F.C. (no stress)], during three different plant growth stages (stem elongation stage (S), flowering stage (F) and stem elongation stage + flowering stage (S+F). To observe the quantity of water and percentage of volumetric water content at field capacity for 5 pots, the weight of pots was taken with and without soil, using a digital weighing balance (Soehnle Professional 10 kg max.). For 24 hours pots with soil were submerged in water to saturate the soil, after that pots were taken out of the water, to measure the weight and the volumetric

moisture content by using a theta probe (Prop Type HH2, Delta-T Devices Ltd, Burwell, UK, Plate 2). The weight and the volumetric moisture content were recorded at 2 hour intervals for the first 12 hours, then at 24 and 48 hours when soils had stopped losing weight. Application of water to each pot was done three times weekly to determine the required amount of water to be applied using the soil moisture meter Theta Prop. In this way, pots with a soil moisture reading of volumetric water content below the WS1, WS2, and WS3 and WS4 levels were balanced by adding the required amount of water.

6.3.2. Plant sampling and processing

Fresh *Brassica napus* leaves, stems and roots and flowers were collected at the mid flowering stage and transported to the laboratory using dry ice to inactivate endogenous myrosinase enzyme, then placed into separate plastic bags and stored at -80 °C before freeze-drying. Frozen samples were dried using a GVD6/13 MKI freeze dryer, (GIROVAC Ltd, North Walsham, UK) for 6-7 days before being milled to a fine powder in a micro-grinder (Retsch GmbH Cyclone Mill-Twister, Haan, Germany). Each milled sample was placed in a separate plastic bags and stored below -18°C until required for water extracts preparation and glucosinolates analysis.

6.3.3. Preparation of water extracts

Water extracts were prepared from each plant parts under three water WS1, WS2, and WS3 collected at the mid flowering stage as previously described in chapter 4 (section 4.3.3). The sample from the treatment WS4= 100% of F.C. (no stress) was accidentally discarded by a member of laboratory staff.

6.3.4. Bioassay

Seeds (250) of test weeds [*Phalaris minor* (Retz.) (canary grass), *Convolvulus arvensis* (L.) (field bindweed) and *Sorghum halepanses* (L.) (Johnsongrass)] were prepared as previously described (Chapter 2, section 2.2.2).

Ten ml of extract solution from each plant parts prepared as described in (section 6.3.3) was added to each petri dish and distilled water was used as the control. All Petri dishes were placed randomly in plant growth chambers (Sanyo MLR) at 25°C in dark conditions. Treatments were arranged in a completely randomized design (CRD) with factorial arrangements in six replications (3 levels of water stress x 3 plant growth stages) for each weed species separately.

6.3.5. Assessment

Germination, shoot and root length and fresh weight of seedlings was measured as as previously described (Chapter 2, section 2.2.3).

6.3.6. Determination of glucosinolate

The different glucosinolates and myrosinase activity were determine in *Brassica napus* as previously described in Chapter 4 (see section 4.3.6 to section 4.3.7).

6.3.7. Statistical analysis

The experiment consisted of two factors (3 water stress x 3 plant growth stage) with five replicates treatments arranged in a completely randomized design for each weed speceis.Two-way ANOVA (water stress x plant growth stage) was carried out to analyse the experiment results using GenStat® 15th Edition (VSN international, Hemel Hemstead, UK) for each palnt part and weed species separately.

Also, two –way ANOVA was undertaken to analyse the results of Glucosinolatet concetrations and myrosinase activity using GenStat® 15th Edition (VSN international, Hemel Hemstead, UK) (water stress x plant growth stage) with five replications for each plant part separately. Where necessary, data were log10-transformed to normalise residuals. A Duncan multiple range test was used to compare the differences between means of treatments at level ($P < 0.05$).

6.4. Results

6.4.1. Bioassay

6.4.1.1. Seed germination %

The results showed that seed germination % of *Phalaris minor* , *Convolvulus arvensis* and *Sorghum halepanses* was significantly ($P > 0.05$) inhibited by applying water extracts from different parts of *Brassica napus* under all water stress levels and during different plant growth development stages Figures 6.1 A, B, C and D.

Applying flower and stem water extract treatments under all water stess levels during all plant development stages was found to be more effective on the seed germination of all three weed species as compared with root and leaf water extracts. However, *C. arvensis* seed germination (%) was significantly reduced by leaf water extacts under all three water stress levels when applied during stem to flower plant development stages (Figure 6.1 D). As shown in Figure 6.1 B, the most effective treatment on seed germination (%) of *C. arvensis* and *S. halepanses* was when the brassica plant was under water stress (30% of field capacity) (WS1) during stem elongation + flowering stage, however, *P. minor* seed germination % was most affected by all three water stress treatments during the flowering stage. Meanwhile, *P. minor* and *C. arvensis* seed germination % was highly suppressed by applying

roots water extract under water stress treatment (70% of fid capacity) (WS3) during stem elongation + flowering stage (Figure 6.1 C).

6.4.1.2. Shoot length (cm)

The effect of all factors using water extracts from different parts of *B. napus*, different levels of water stress during different plant development stages on shoot length of *P. minor* (Retz.), *C. arvensis* (L.) and *S. halepense* (L.) is presented in figures 6.2 A, B C and D. The results of data analysis showed that water extracts from all parts of *B. napus*, different water stress levels during different plant development stages significantly (p<0.001) supressed shoot length of all three weed species (Figures 6.2 A, B C and D). The greatest inhibition of shoot length of *P. minor* and *C. arvensis* was found when flower water extract was applied to plants under water stress (30% of field capacity) (WS1) during stem elongation. While applying flower water extract under water stress (70% of field capacity) (WS3) during stem elongation and flowering stage S+F the water extract appeared to have the greatest effect on shoot length of *S. halepanses* (Figure 6.1 A). However, under water stress, WS1, during S+F the highest inhibition of *C. arvensis,* shoot length was revealed when stem water extract was applied (Figure 6.1 B). Whereas, *P. minor* shoot length was significantly reduced by applying stem water extract under water stress WS2 during F. However, *S. halepanses* shoot length was greatly affected under water stress WS3 during S+F. Meanwhile, root water extract tended to be the most effective on shoot length of *P. minor* under water stress WS1 during F and shoot length of *S. halepanses* during S+F. However, shoot length of *C. arvensis was* effected under water stress WS3 during F (Figure 6.1 C). On the other hand, water stress WS1 during S+F treatment showed that the highest application of root extract reduced *P. minor* and *S. halepanses* shoot length significantly. Water

stress WS3 during F treatment revealed a significantly higher reduction in shoot length of *C. arvensis* (Figure 6.1 D).

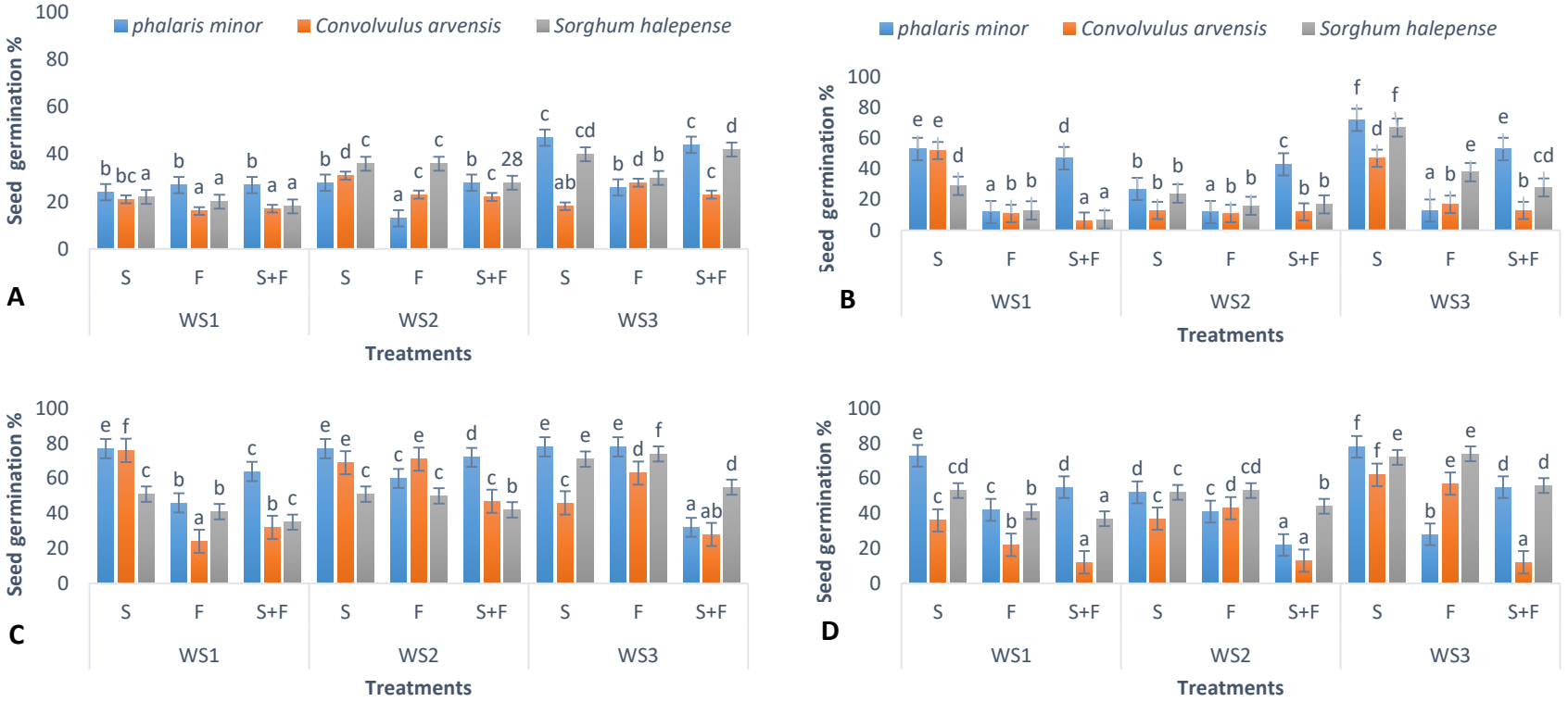

Figure 6.1. Effect of water extract from different *B. napus* parts (A) flowers, (B) stems, (C) roots and (D) leaves at different water stress levels [WS1=30% of field capacity (F.C.), WS2= 50% of F.C. and WS3= 70 % of F.C.] at different plant growth, stages stem elongation stage (S), flowering stage (F) and stem elongation stage + flowering stage (S+F) on the seed germination % of weed species after 14 days. Bars with the same letter are not significantly different according to Duncan's multiple range test ($P < 0.05$). Error bars represents standard error of means. n = 45 for each treatment. n = 45 for each treatment.

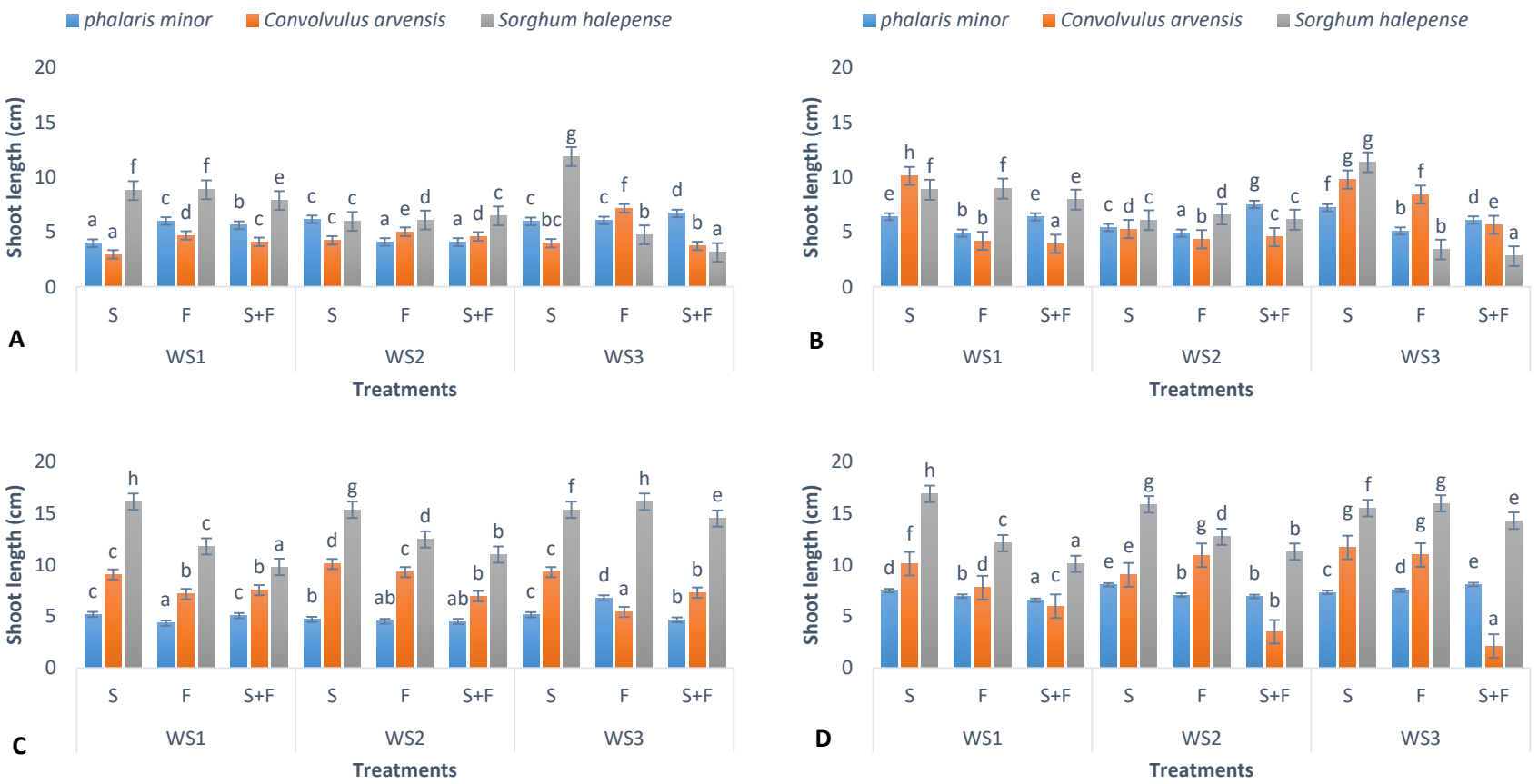

Figure 6.2. Effect of water extract from different *B. napus* parts (A) flowers, (B) stems, (C) roots and (D) leaves at different water stress levels [WS1=30% of field capacity (F.C.), WS2= 50% of F.C. and WS3= 70 % of F.C.] at different plant growth, stages stem elongation stage (S), flowering stage (F) and stem elongation stage + flowering stage (S+F) on the shoot length (cm) of weed species after 14 days. Bars with the same letter are not significantly different according to Duncan's multiple range test ($P < 0.05$). Error bars represents standard error of means. n = 45 for each treatment.

6.4.1.3. Root length (cm)

The results of data presented in figures 6.3 A, B, C and D revealed that water extract from all *B. napus* plant parts under different levels of water stress (WS1, WS2 and WS3) during different plant development stages S, F and S+F significantly (P<0.001) inhibited root length of *Phalaris minor* (Retz.), *Convolvulus arvensis* (L.) and *Sorghum halepense* (L.). The results show that water extracts from flowers tends to be a more effective treatment for root length for all three weed species under all water stress conditions and during all plant development stages, followed by stems extracts and then leaf extracts (Figures 6.3 A, B and D). However, leaf extract under WS3 during S trend to be more effective than stem extract under WS3 during S on all three weed species (Figures 6.3 C and D). In general, the greatest inhibitor to root length of *P. minor* was found in the Petri dish treated with flower extract. Root length was less than 1 cm under all water stress levels and during all plant development stages as compared with *C. arvensis* root length in treatments and *S. halepense* under WS1 and WS3 during S plant development stage (Figures 6.3 A). However, no root length changes were reported with *S. halepense* seedling when treated by flower and stem extract under WS1, during S+F plant development stage, WS2 under S and S+F and WS3 under F and S+F (Figures 6.3 A and B). The greatest values of reducing *C. arvensis* root length were found when treated with leaf extract under WS1 during F and S+F, also under WS2 and WS3 under S+F (Figure 6.3 D). The observations in figure 6.3 C revealed that there was no root length change for *S. halepense* seedlings when treated with root extract under WS1 during S+F.

6.4.1.4. Shoot fresh weight (mg)

The effect of using water extracts from different parts of *B. napus* under different water stress levels and all plant development stages and their interactions on shoot fresh weight of *P. minor* (Retz.), *C. arvensis* (L.) and *S. halepense* (L.) is shown in figures 6.4 A, B, C and D.

The results of data analysis showed that water extracts from different parts of *B. napus* under different water stress levels and during all plant development stages significantly (p<0.001) inhibited shoot fresh weight of *P. minor* (Retz.), *C. arvensis* (L.) and *S. halepense* (Figures 6.4 A, B, C and D). Shoot fresh weight of *P. minor* and *S. halepense* was significantly inhibited and tended to be more affected by water extracts from all plant parts under all water stress levels during all plant development stages as compared with *C. arvensis* (Figures 6.4 A, B, C and D). In the case of applying leaf extract under WS2 and WS3 during S+F the results show that shoot fresh weight of *C. arvensis* was significantly supressed compared with the other shoot fresh weight of weed species (Figurer 6.4 D). Shoot fresh weight of *S. halepense* showed more sensitivity to the water extract from flowers and roots under all treatments (Figures 6.4 A and C). However, it is less effected by leaf extract under all treatments (Figures 6.4 D).

6.4.1.5. Root fresh weight (mg)

The results revealed that *P. minor* (Retz.), *C. arvensis* (L.) and *S. halepense* root fresh weight was significantly (P<0.05) affected by water extract from flowers, stems and roots of *B. napus* under different levels of water stress and during all plant development stages as illustrated in figures 6.5 A, B and C.

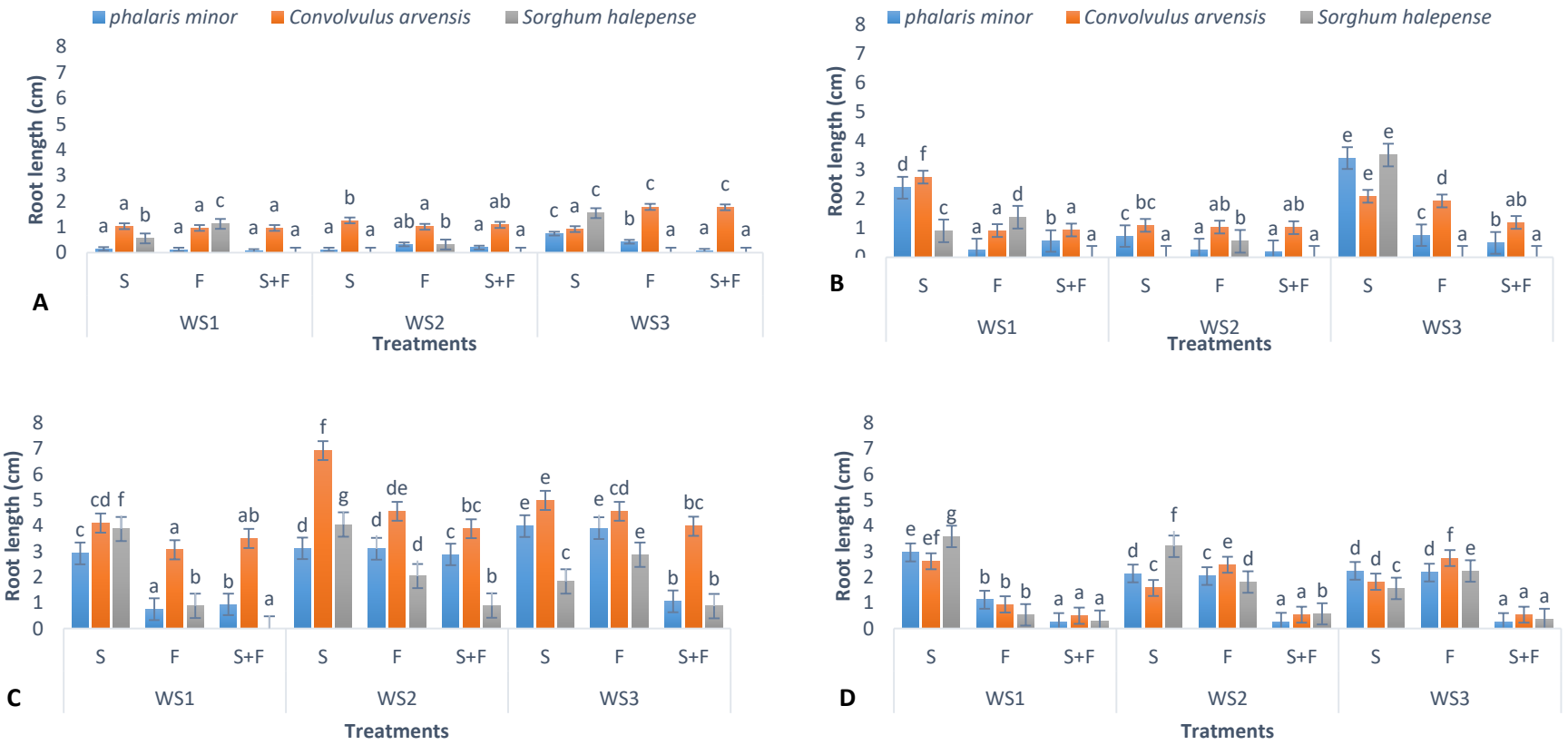

Figure 6.3. Effect of water extract from different B. napus parts (A) flowers, (B) stems, (C) roots and (D) leaves at different water stress levels [WS1=30% of field capacity (F.C.), WS2= 50% of F.C. and WS3= 70 % of F.C.] at different plant growth, stages stem elongation stage (S), flowering stage (F) and stem elongation stage + flowering stage (S+F) on the root length (cm) of weed species after 14 days. Bars with the same letter are not significantly different according to Duncan's multiple range test ($P < 0.05$). Error bars represents standard error of means. n = 45 for each treatment.

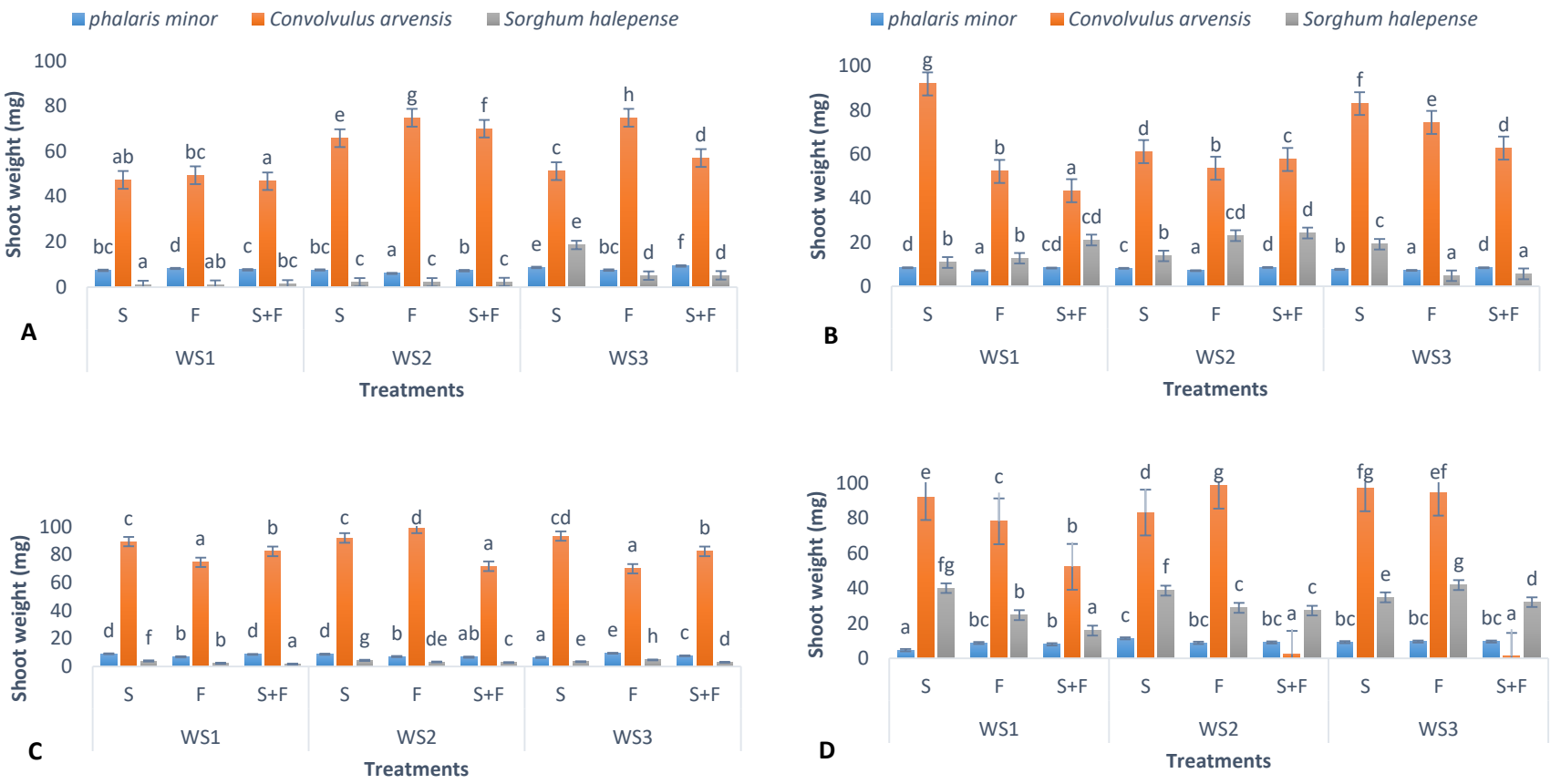

Figure 6.4. Effect of water extract from different *B. napus* parts (A) flowers, (B) stems, (C) roots and (D) leaves at different water stress levels [WS1=30% of field capacity (F.C.), WS2= 50% of F.C. and WS3= 70 % of F.C.] at different plant growth, stages stem elongation stage (S), flowering stage (F) and stem elongation stage + flowering stage (S+F) on the shoot weight (mg) of weed species after 14 days. Bars with the same letter are not significantly different according to Duncan's multiple range test ($P < 0.05$). Error bars represents standard error of means. n = 45 for each treatment.

Additionally, leaf extract inhibited *C. arvensis* (L.) and *S. halepense* root fresh weight was significantly reduced ($P<0.001$) under different levels of water stress and during all plant development stages. While *P. minor* root fresh weight was reduced significantly during all plant development stages. Moreover, no significant effect of water stress was found on *P. minor* root fresh weight (Figure 6.5 D). The observations showed that root fresh weight of *P. minor* and *S. halepense* tend to be more effected by flower and stem water extract under all water stress levels and during all plant development stages (Figures 6.5 A and B). As described previously (see section 6.4.1.3), no root length changes were reported with *S. halepense* seedlings when treated with flower and stem extract under WS1 during S+F plant development stage, WS2 under S and S+F and WS3 during F and S+F (Figures 6.3 A and B). Meanwhile, the greatest inhibition of root fresh weight of *C. arvensis* was found when treated with flower extract under water stress level WS1 under F and S+F and with applying stems extract under WS1 during S+F, also, when treated by root extract under SW1 during F and S+F and WS2 during F (Figure 6.3 C). Leaf extract was more effective however, on root fresh weight of *C. arvensis* under WS3 during S+F (Figure 6.3 D).

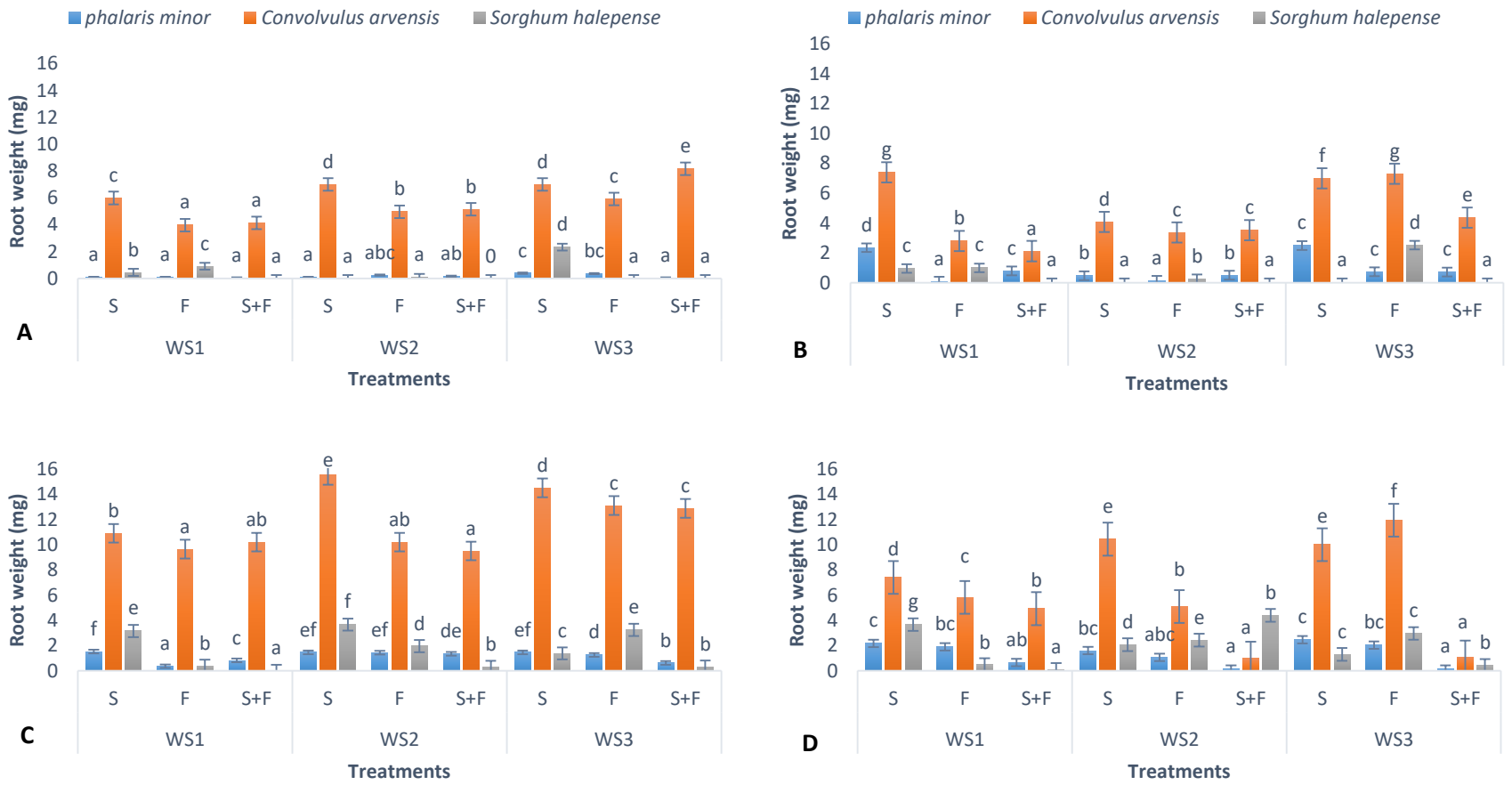

Figure 6.5. Effect of water extract from different *B. napus* parts (A) flowers, (B) stems, (C) roots and (D) leaves at different water stress levels (WS) [WS1= 30% of field capacity (F.C.), WS2= 50% of F.C. and WS3= 70 % of F.C.] at different plant growth, stages stem elongation stage (S), flowering stage (F) and stem elongation stage + flowering stage (S+F) on the root weight (mg) of weed species after 14 days. Bars with the same letter are not significantly different according to Duncan's multiple range test ($P < 0.05$). Error bars represents standard error of means. n = 45 for each treatment.

6.4.2. Individual and total glucosinolates concentrations

In this study 10 individual glucosinolates (GSL) from of *B. napus* parts were found; Progoitrin, gluconapoleiferin, gluconapin, glucobrassinapin, glucoraphanin, glucotropaeolin, glucobrassicin, 4OH glucobrassicin, neoglucobrassicin and gluconasturtiin by using HPLC analysis.

6.4.2.1. Effect of water stress levels during different plant development stages on individual glucosinolates concentration in different parts of *B. napus*.

The effect of water stress levels during different plant development stages on concentration of individual glucosinolates in different *B. napus* parts is demonstrated in figures 6.6, 6.7, 6.8 and 6.9. There was a great difference in the concentration of glucosinolates between the different plant parts under different water stress levels and plant growth stages. Progoitrin, glucoraphanin, gluconapin, and glucobrassinapin concentrations in flower tissue were significantly ($P< 0.05$) affected by all treatments. However, gluconasturtiin it not present in flower tissue (Figures 6.6). Progoitrin concentration significantly ($P< 0.05$) increased in flower tissue as compared with the other glucosinolates concentration under all water stress levels and during all plant development stages. The greatest value obtained was under WS2 during F, followed by WS3 during S (Figures 6.6). Also, the glucobrassinapin concentration was higher as compared with glucoraphanin, gluconapin under all treatments, followed by glucoraphanin then gluconapin (Figures 6.6). In general, the concentration of progoitrin, glucoraphanin, gluconapin, and glucobrassinapin in flower tissues was significantly higher. The results in figure 6.7 shows there was significant ($P< 0.05$) difference in concentration of Progoitrin, glucoraphanin, gluconapin, and glucobrassinapin under different water stress levels

and different plant growth stages in *B. napus* leaves tissues. However, the concentration of all glucosinolates was low. The progoitrin concentration was greater than the other especially under SW2 during S, F and S+F, followed by glucobrassinapin concentration. Also, in stem tissue the observations revealed that progoitrin concentration was highest when compared with the other glucosinolates under all water stress levels and during all plant development stages treatment. The greatest value of progoitrin concentration was determined by WS1 during S+F and WS3 during S (Figure 6.8). The next highest glucosinolate concentration was glucobrassinapin when plants were under water stress levels WS3 during S. while, gluconasturtiin concentration was the lowest. However, in root tissue the gluconasturtiin concentration recorded the highest value and significantly increased compared with the other glucosinolates concentration under all water stress levels and during all plant development stages (Figure 6.9), the next best glucosinolates in concentration was progoitrin.

6.4.2.2. Effect of water stress levels during different plant development stages on total glucosinolates concentration in different parts of *B. napus*

There was significant (P<0.05) difference in concentration of total glucosinolate in various parts of *Brassica* napus under all water stress levels and different development stages (as revealed in figure 6.10), and both factors significantly affected total glucosinolates. Total glucosinolates concentration in roots under water stress level WS2 during plant development stages S and S+F was significantly (P<0.001) higher as compared with total glucosinolates concentration under the other treatments (Figure 6.10). Meanwhile, the total glucosinolates concentration in flower extract was significantly increased under water stress WS2 and WS3 during plant development F. Also, the total glucosinolates concentration in stem extract increased significantly and a higher concentration reported under water stress level

WS3 under plant development stages S, followed by SW2 under S+F treatment (Figure 6.10). Whereas, the total glucosinolates concentration in leaves extract was significantly lower compared with the other parts. The highest total glucosinolates concentration in leaf extract was reported under water stress level WS3 during plant development F as compared with the other treatments (Figure 6.10).

6.4.3. Effect of water stress levels during different plant development stages on myrosinase enzyme activity in different parts of *B. napus*

There were significant ($P > 0.05$) differences in activity of myrosinase enzyme between different *B. napus* plant parts, water stress levels and plant development stages (Figure 6.11). Myrosinase activity significantly increased in flowers and leaf extract at different plant growth stages. There were no significant differences found between the treatments on myrosinase activity in flower extract. However, significant differences between treatments were observed on myrosinase activity in roots extract (Figure 6.11). The greatest value of myrosinase activity in flower extract was found under WS1 during S stage and WS2 during F stage. In root extract the highest myrosinase activity was reported when plants were treated under water stress WS2 during plant development stages F and S+F, as compared with all other treatments (Figure 6.11),while, the lowest value was documented in stem and root extract. In stems extract the lowest myrosinase activity was found under WS1 during S+F. Whereas, in root extract, the lowest myrosinase activity was reported under WS2 during S+F (Figure 6.11).

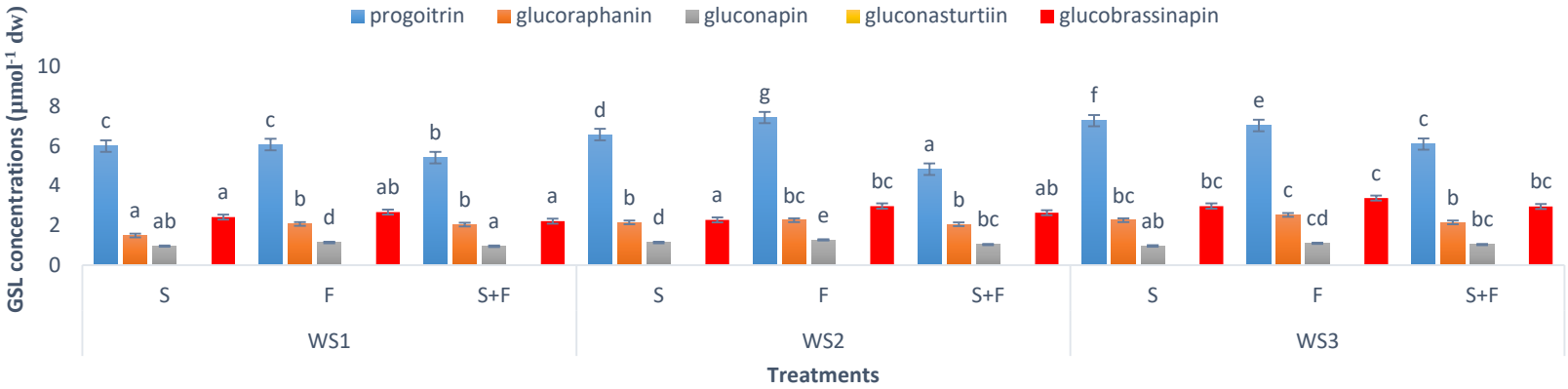

Figure 6.6. Effect of different water stress levels [WS1= 30% of field capacity (F.C.), WS2= 50% of F.C. and WS3= 70 % of F.C.] at different plant growth, stages stem elongation stage (S), flowering stage (F) and stem elongation stage + flowering stage (S+F) on glucosinolates concentration in flowers dry tissue. Bars with the same letter are not significantly different according to Duncan's multiple range test ($P < 0.05$). Error bars represents standard error of means. n = 45 for each treatment.

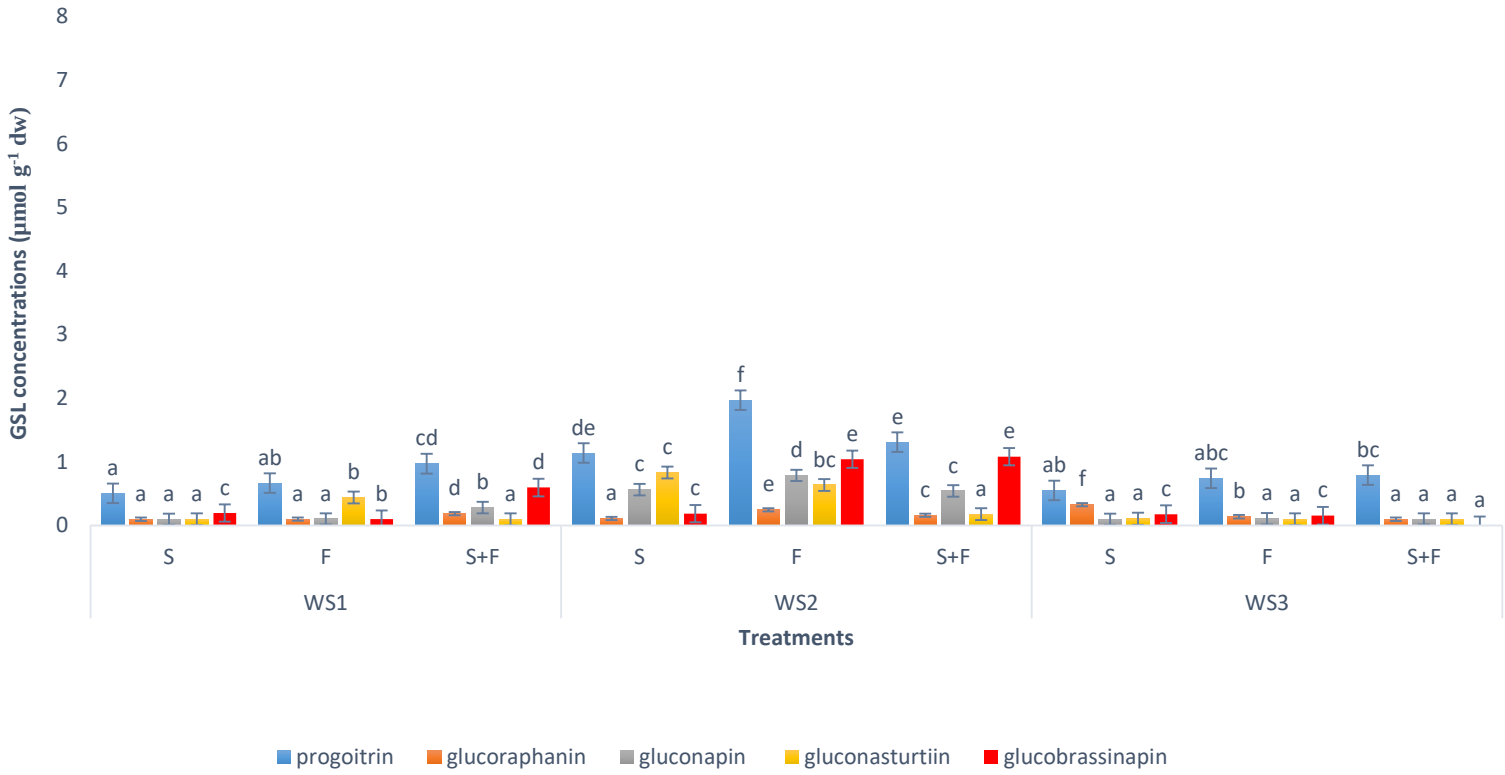

Figure 6.7. Effect of different water stress levels [WS1= 30% of field capacity (F.C.), WS2= 50% of F.C. and WS3= 70 % of F.C.] at different plant growth, stages stem elongation stage (S), flowering stage (F) and stem elongation stage + flowering stage (S+F) on glucosinolates concentration in leaves dry tissue. Bars with the same letter are not significantly different according to Duncan's multiple range test ($P < 0.05$). Error bars represents standard error of means. n = 45 for each treatment.

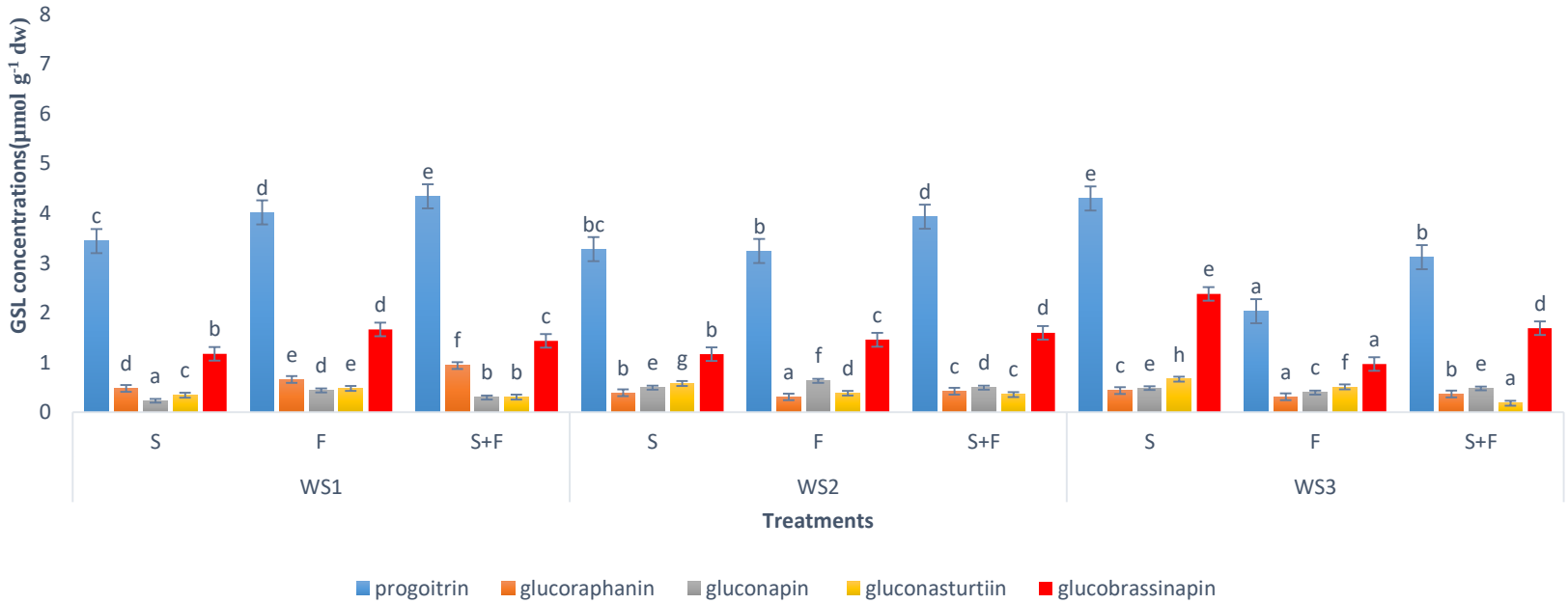

Figure 6.8. Effect of different water stress levels [WS1= 30% of field capacity (F.C.), WS2= 50% of F.C. and WS3= 70 % of F.C] at different plant growth, stages stem elongation stage (S), flowering stage (F) and stem elongation stage + flowering stage (S+F) on glucosinolates concentration in stems dry tissue. Bars with the same letter are not significantly different according to Duncan's multiple range test ($P < 0.05$). Error bars represents standard error of means. n = 45 for each treatment.

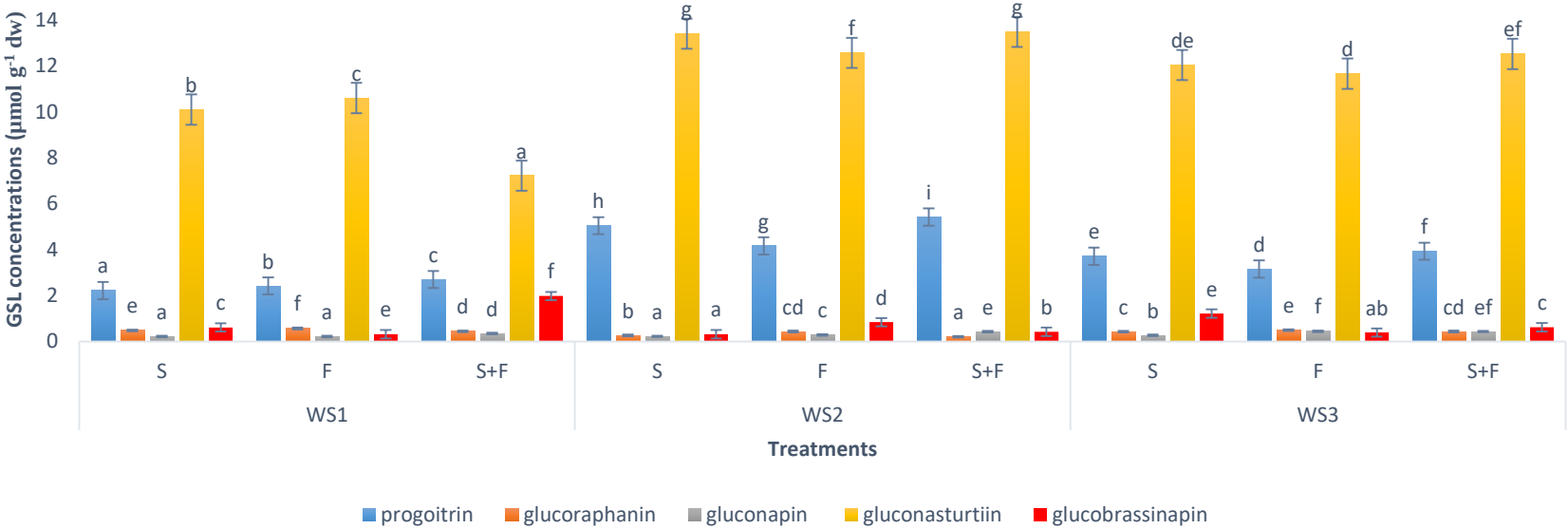

Figure 6.9. Effect of different water stress levels [WS1= 30% of field capacity (F.C.), WS2= 50% of F.C. and WS3= 70 % of F.C.] at different plant growth, stages stem elongation stage (S), flowering stage (F) and stem elongation stage + flowering stage (S+F) on glucosinolates concentration in roots dry tissue. Bars with the same letter are not significantly different according to Duncan's multiple range test ($P < 0.05$). Error bars represents standard error of means. n = 45 for each treatment.

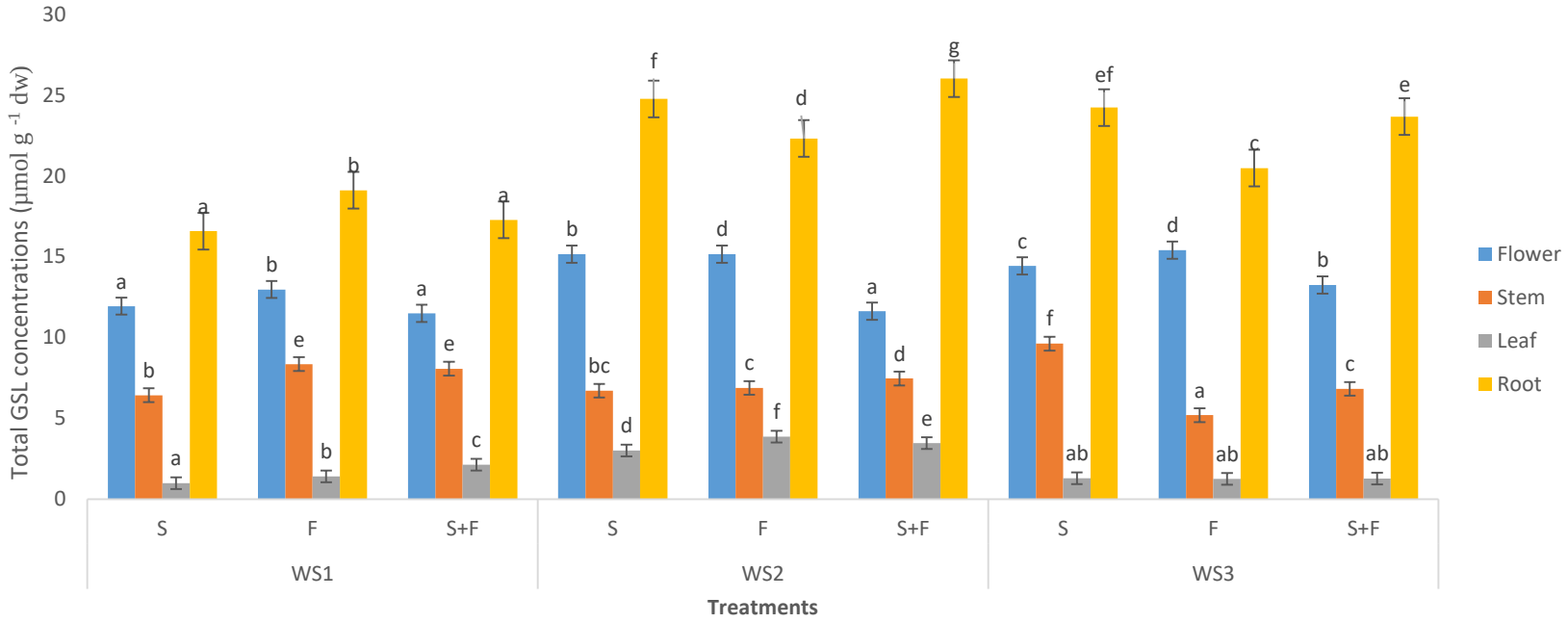

Figure 6.10. Effect of different water stress levels [WS1= 30% of field capacity (F.C.), WS2= 50% of F.C. and WS3= 70 % of F.C.] at different plant growth stages stem elongation stage (S), flowering stage (F) and stem elongation stage + flowering stage (S+F) on total glucosinolates concentration in different *B. napus* parts. Bars with the same letter are not significantly different according to Duncan's multiple range test ($P < 0.05$). Error bars represent standard error of means. n = 45 for each treatment.

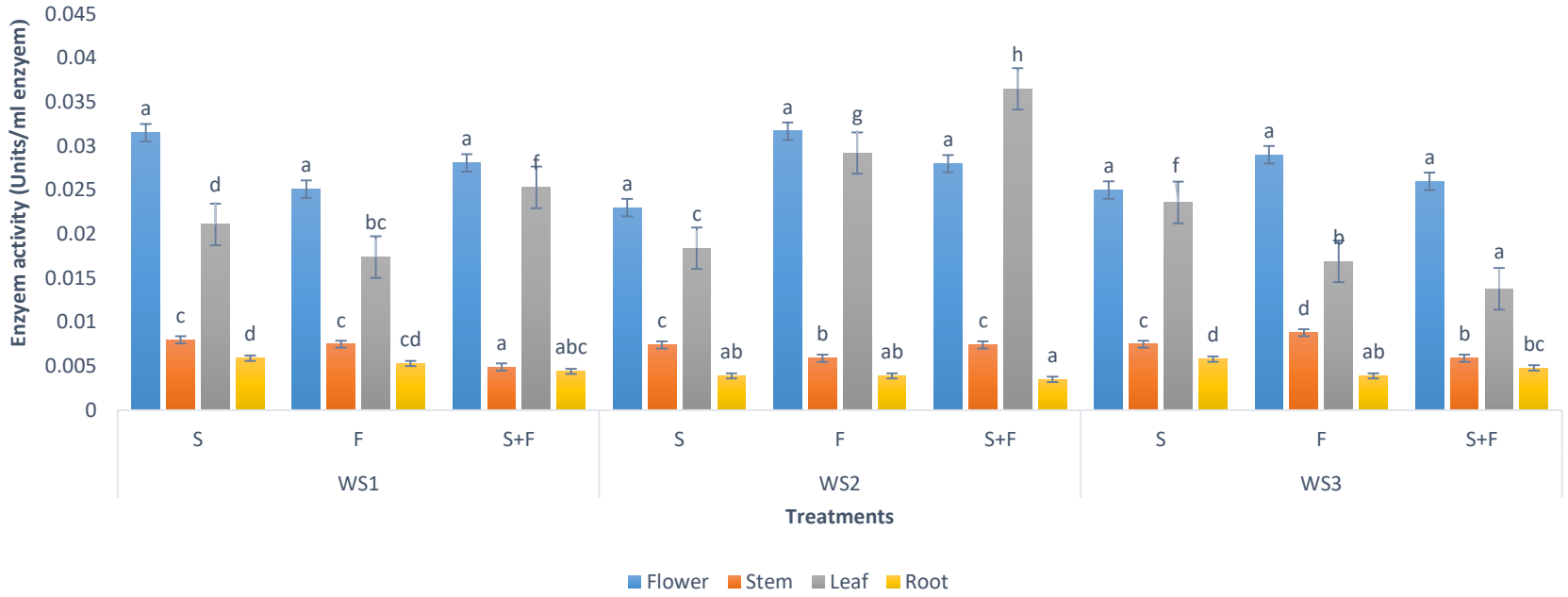

Figure 6.11. Effect of different water stress levels [WS1= 30% of field capacity (F.C.), WS2= 50% of F.C. and WS3= 70 % of F.C.] at different plant growth stages, stem elongation stage (S), flowering stage (F) and stem elongation stage + flowering stage (S+F) on myrosinase enzyme activity in different *B. napus* parts. Bars with the same letter are not significantly different according to Duncan's multiple range test ($P < 0.05$). Error bars represents standard error of means. n = 45 for each treatment.

6.5. Discussion

In this study, an in *vitro* experiment was carried out to examine the effects of water extract from different *B. napus* parts under different water stress levels (WS1, WS2 and WS3) and during different plant development stages (S, F and S+F) on germination and seedling growth of weed species; *Phalaris minor* (Retz.), *Convolvulus arvensis* (L.) and *Sorghum halepense*. Also, their effect on individual and total glucosinolates

6.5.1 Weeds species germination and seedling growth

6.5.1.1 Weeds species germination

The water extract from different parts of *B. napus* during the three water stress levels under all plant development stages studied demonstrated variability in their effect on germination and seedling growth of three weed species *P. minor* (Retz.), *C. arvensis* (L.) and *S. halepense* (L.) between the water stress levels and within the same plant development stage (Tang *et al*., 1995; Karageorgou *et al*., 2002; Szabó *et al*., 2003; Gray *et al*., 2003; Tawaha and Turk, 2003; Wakjira *et al*., 2005; Uremis *et al*., 2009; Taiz and Zeiger 2010).

This finding showed that seed germination % of all three weed species significantly decreased by treating with water extract from all parts of *B. napus* during the three water stress levels under all plant development stages as compared with the control from previous results observed in chapters 1, 2 or 3. However, the effect of *B. napus* plant part extracts on weed species seed germination percentage was varied. These findings are in line with those reported by (Chon and Kim, 2002; Turk and Tawaha, 2002; Turk and Tawaha; 2003).

The results were similar with those observed in chapter 4. The greatest values of *P. minor* germination% reduction was found when treated with flower extract under WS2 during F and stems extract WS1, and WS3 during F, as compared with leaf and root extract. These observations contradicted results found by Turk and Tawaha (2003). They found that wild barley (*Hordeum spontaneum*) germination was most effective when treated by water extract from black mustard leaves, .The contrast might be due to using water extracts from different *Brassica spp* on different weed species in their experiment. Furthermore, the effects between different levels of water stress during different plant development stages on weed species germination were varied, and is more likely to be due to the differences among individual and total glucosinolates concentration found in *B. napus* under different water stress levels at different plant development stages. These results are in line with data observed by Brown *et al*., 2003; Redovniković *et al*., 2008; Khan *et al*., 2010; Ullah *et al*., 2012 and Martinez-Ballesta *et al*., 2013, who documented differences between the glucosinolate profiles and their concentrations in the different plant parts under different water stress levels and during different plant developmental stages. Moreover, seed germination of *C. arvensis* (L.) and *S. halepense* was more sensitive to stem extract under water stress WS1 during plant development S+F. These results may be due to high concentration of progoitrin and total glucosinolates in flower extract during the flowering stage. These findings are in line with those reported by (Peterson *et al*., 2001; Malik *et al*., 2010; Modhej *et al*., 2013). These authors reported that the inhibition in weed germination by *Brassica spp*. was probably due to isothiocyonamatic compounds which had a high ability to suppress seed germination. Similarly, seeds germination of *Convolvulus arvensis* (L.) was very sensitive to leaf extract under all water stress levels during plant development stage S+F. Although, the total glucosinolates was highest in the root tissues, roots water extract was the least effective on all weed species under all water stress levels

and during all plant development stages. This result may be because of the activity of myrosinase being lower than other plant parts (Bennett and Wallsgrove, 1994; Wittstock and Gershenzon, 2002).

6.5.1.2. Weed species shoot and root length

In three weed species *Phalaris minor* (Retz.), *Convolvulus arvensis* (L.) and *Sorghum halepense* (L.), applying water extract from all *B. napus* parts under all water stress levels during different plant development stages have been shown to reduce the shoot and root length. Shoot and root length of all three weed species in the present study appeared to be more sensitive to flower and stem water extract as compared with leaf and root water extract (Abdel-Farid *et al*., 2014). However, Turk and Tawaha (2003) disagree with these results, as they found that leaf extract was most effective on weeds' shoot and root length. In general, the shoot length of *P. minor* (Retz.) and *C. arvensis* was shorter following exposure to each type of water extract regardless of the treatment factors, whereas the shoot length of *S. halepense* was specifically affected by applying flower water extract under water stress (WS3) during S+F. This might be due to different water stress treatments increasing the glucosinolate accumulation in flower and stem tissues compared with the concentration in leaf and root tissues. Hence, the decrease in shoot length of weed species may relate to the phytotoxic effect of glucosinolates hydrolysis products, observed in *B. napus* flower and stem tissues. This result is in keeping with the findings of Chung and Miller (1995), and Turk and Tawaha (2003). Furthermore, shorter shoot length of *P. minor* and *C. arvensis* was obtained when treated by flower extracts under WS1 during S. Further, *C. arvensis* shoot length was reduced significantly by leaf extract under WS3 during S+F. Moreover, water extract from flower and stem under SW3 during S+F was the most effective treatment on *S. halepense* shoot length. On the other hand, root length of all weed

species was affected more than that of the shoot length. These findings are in line with earlier observations that plant water extract had greater toxic effects on root length than on shoot length (Chung and Miller, 1995; Tawaha and Turk, 2003; Turk and Tawaha, 2003; Turk *et al*. 2005). These out comes might be because roots are the first to imbibe the allelochemicals from the water extracts (Jenning and Nelson, 2002; Turk and Tawaha 2002), or it could be as a result of direct contact of root with the water extract and thus with biocidal chemical compounds (Quasem 1995). In addition to the root length inhibition by different water extracts, weed root morphology was deformed such as root twisting (Jenning and Nelson, 2002; Matloob *et al*., 2010). Moreover, the root of *S. halepense* also was severely stunted when applied with stem extract under WS1 during S+F, WS2 during S and S+F and WS3 during F and S+F. In general, root extract under different WS levels and during different plant development stages was the less effective on weed species root length.

6.5.1.3. Weed species shoot and root fresh weight

In the this experiment, the shoot and root fresh weight of all three weed species was significantly inhibited by treating with water extracts from all *B. napus* parts under different water stress levels and during different plant growth development. *P. minor* and *S. halepense* shoot fresh weight seemed to be the most effective treated with water extracts from all *B. napus* parts under different water stress levels and during different plant growth development treatments. The effects change from one treatment to another because of the changes in individual and total glucosinolates concentration and myrosinase activity under the same treatment (Keling and Zhujun, 2010). In this study, the highest inhibition in *P. minor* shoot fresh weight was when treated with flower and leaf water extract (Tawaha and Turk, 2003; Turk and Tawaha, 2003; Abdel-Farid *et al*., 2014). Leaf extract under WS1 during S

had a great effect on *C. arvensis* shoot fresh weight and was significantly reduced under WS2 and WS3 during S+F.

Additionally, flower, stems and roots extract significantly supressed *P. minor* and *S. halepense* root fresh weight more than *C. arvensis* root fresh weight. Furthermore, when *S. halepense* was treated with flower extract under WS1 during S+F, WS2 during S and S+F and WS3 during F and S+F no root changes were recorded. Also, when stem extract was applied under WS1 during S+F, WS2 and WS3 during S and S+F, results showed that the treatments vary in their effect on *S. halepense* root fresh weight. This finding may due to the high concentration of progoitrin and glucobrassinapin in flower, stem and root extract. Moreover, root fresh weight was the least effected by all treatments. The outcomes showed that root fresh weight was affected by all treatments more than that of the shoot fresh weight (Quasem 1995).

6.5.2. Individual, total glucosinolates and myrosinase activity

In the present study, the concentration and type of individual glucosinolates in *B. napus* parts (stems, leaves, roots and flowers) and also within the parts under different water stress levels and during different plant development stages. All glucosinolates obtained in this study were significantly affected by water stress levels. On the other hand, similar results obtained for total concentration of glucosinolates were affected by *B. napus* parts under all treatments. These observations support previous work (Rosa *et al*., 1996; Champolivier and Merrien, 1996; Kirkegaard & Sarwar, 1998; Lambdon *et al*., 2003; Bellostas *et al*., 2007). However, the results contradict data observed by Radovich *et al*. (2005), who found that glucosinolate concentration was not affected by plant development stages. Ten individual glucosinolates were reported in different parts of *B. napus* in the present experiment; Progoitrin, gluconapoleiferin, gluconapin, glucobrassinapin,

glucoraphanin, glucotropaeolin, glucobrassicin, 4OH glucobrassicin, neoglucobrassicin and gluconasturtiin. Based on the concentrations Progoitrin, glucoraphanin, gluconapin and gluconasturtiin and glucobrassinapin were dominate and the highest glucosinolates observed. Progoitrin recorded the greatest value in flower, stem and leaf extract under all treatments, followed by glucobrassinapin. However, in root extract, gluconasturtiin was reported to have the highest concentration followed by progoitrin. In general, glucosinolates concentration slightly increased in WS2 and SW3 as compared with WS1. These results support previous data recorded by Khan *et al*. (2010). Furthermore, total glucosinolate was significantly affected by water stress and plant development stages in different plant parts. The highest total glucosinolates was observed in *B. napus* roots extract under all water stress levels and during all plant development stages. This increase in the total glucosinolate in root was possibly because of the increase in concentration of gluconasturtiin. Moreover, total glucosinolate in flower extracts was significantly higher, followed by stem extract and then leaves. As the results reveal flower and stem extract were more effective on weed germination and growth seedling. However, total glucosinolate in root extract was significantly higher. Therefore, this may be due to the concentration of dominate glucosinolates being higher in flower and stem extracts, hence, the relationship between them where high activity of myrosinase could be more effective than gluconasturtiin with high concentrations and low concentrations of other individual glucosinolate in root extract. This finding support the results obtained from previous experiments (Chapter 4and 5) which showed that the dominated glucosinolates are greatly toxic to weed species germination and seedling growth. This observation matches that of Abdel-Farid *et al*. (2014), who found that the glucosinolates in flower extract had high toxicity to weed germination and seedling growth. The glucosinolate- myrosinase system is one defence system against antagonists using Brassica members (Bennett and

Wallsgrove, 1994; Wittstock and Gershenzon, 2002). In this study the results show significant differences in myrosinase activity between different plant parts under different water stress and during different plant growth development sages. In general, the myrosinase activity in flower extracts is significantly higher than in other plant parts under different water stress and during different plant growth development sages, followed by leaf, stem and roots. Although, the enzyme activity was significantly higher in leaf extracts, the effect of water extracts from leaves on weed germination and seedling growth was less. These results support the data reported by Hansen (2011), who found that although differences in soil myrosinase activity were detected among the samples, no positive correlation was found between soil myrosinase activity and glucosinolates concentrations (released after incorporation of freeze-dried mustard leaves).

6.6. Conclusion

The results obtained from this experiment have clearly shown that seed germination % of all three weed species significantly reduced by treating with water extract from all parts of *B. napus* during the all water stress levels under all plant development stages. The highest values of *P. minor* germination% reduction was obsreved when treated with flower extract under WS2 during F and stems extract WS1, and WS3 during F. Additionally, seed germination of *C. arvensis* (L.) and *S. halepense* was more affected by stem extract under water stress WS1 during plant development S+F.

Also, of *P. minor* and *C. arvensis* shoot length was reduced significantl by flower extracts under WS1 during S. Moreover, water extract from flower and stem under SW3 during S+F was the most effective treatment on *S. halepense* shoot length. Root length of all weed species was affected more than that of the shoot length.

Root of *S. halepense* also was significantly reduced when applied with stem extract under WS1 during S+F, WS2 during S and S+F and WS3 during F and S+F.

Shoot fresh weight of *P. minor* and *S. halepense* was the most affected when treated with water extracts from all *B. napus* parts under different water stress levels and during different plant growth development treatments. The highest inhibition in *P. minor* shoot fresh weight was when treated with flower and leaf water extract. Leaf extract under WS1 during S had a great effect on *C. arvensis* shoot fresh weight and was significantly reduced under WS2 and WS3 during S+F.

Flower, stems and roots extract significantly supressed *P. minor* and *S. halepense* root fresh weight more than *C. arvensis* root fresh weight. Furthermore, when *S. halepense* was treated with flower extract under WS1 during S+F, WS2 during S and S+F and WS3 during F and S+F no root changes were recorded. Also, when stem extract was applied under WS1 during S+F, WS2 and WS3 during S and S+F, results showed that the treatments vary in their effect on *S. halepense* root fresh weight.

Glucosinolates concentration slightly increased in WS2 and SW3 as compared with WS1. The highest total glucosinolates was observed in *B. napus* roots extract under all water stress levels and during all plant development stages. Moreover, total glucosinolate in flower extracts was significantly higher, followed by stem extract and then leaves. Flower and stem extract were more effective on weed germination and growth seedling. Progoitrin recorded the greatest value in flower, stem and leaf extract under all treatments, followed by glucobrassinapin. In general, the myrosinase activity in flower extracts is significantly higher than in other plant parts under different water stress and during different plant growth development sages, followed by leaf, stem and roots.

Chapter 7

7. General Discussion

7.1. General Discussion

7.1. General Discussion

Prior this research, there has been little information available on the effectiveness of the *Brassica napus* for weed species inhibition (*Phalaris minor* (Retz.), *Convolvulus arvensis* (L.) and *Sorghum halepense* (L.) in the Iraq and Kurdistan region. The aim of the present study is to establish the allelopathic potential in *Brassica napus* and its effect on weed species inhibition in glasshouse and laboratory settings, and to determine whether other factors such as plant growth stages and water stress levels are involved to increase the allelopathic potential in *B. napus* through increasing the glucosinolate concentrations. This main target was successfully achieved through several experiments reported in chapters 2 to 6.

In the present study, the evaluation has been performed through an initial finding of the allelopathic effect of *Brassica napus* water extract from different plant parts with different concentrations, as described in Chapter 2 and 3. The effect of *Brassica napus* water extracts of different concentrations on weed management is well researched (Al-Khatib *et al.*, 1997; Al-Khatib and Boydston, 1999; Branca *et al*., 2002; Jafariehyazdi and Javidfar, 2011; Yasumoto *et al*.; 2011). However, little attention has been paid in Iraq and Kurdistan region to the use *Brassica napus* water extracts for weed management.

The effect of *Brassica napus* water extracts from different parts on germination and seedling growth of *Phalaris minor*, *Convolvulus arvensis* and *Sorghum halepense* was investigated. Significant results were observed by using *Brassica napus* water extracts from different parts on all three weed species, as seed germination and seedling growth were inhibited in comparison with the control. These observations are in the line with other results reporting that *Brassica spp* water extracts had an

effect on the seed germination and seedling growth of weed species such as canarygrass (*Phalaris minor* Retz), wild oat (*Avena fatua* L.), broad leaf dock (*Rumax dentatus* L.), lambsquarters (*Chenopodium album* L.) and field bind weed (*Convolvulus arvensis* L.) (Al- Khatib and Boydston, 1999; Narwal, 2001; Cheema *at el.*, 2003; Turk and Tawaha, 2003). Moreover, Mason-Sedun *et al*. (1986) found that extracts from *B. nigra* were toxic to wheat growth. Also, Oleszek (1987) observed similar results as it was found that the germination of lettuce, barnyard grass and wheat was inhibited when treated with volatiles from *B. nigra*. Several of these authors refer to the significant effect of numerous *Brassica* species on weed management to possibility of containing high levels of glucosinolate which is able to inhibit germination and supress seedling growth.

Although, the results of this study showed significant effect of water extract from different plant parts on weeds parameters in the laboratory, conclusive proof is often lacking. Numerous studies have obtained different results under laboratory and field conditions (Inderjit & Weston, 2000). In the field, several factors are interacting in simultaneously and sequentially and constantly changing such as temperature, light, aeration, water content of soil, nutrient, soil texture, organic matter, soil microbes and pH of soil. On the other hand, various studies showed ample evidence that allelochemical in soil can be broken down by microbes and that levels usually, although not always, decline rapidly due to microbial degradation (Von Kiparski, Lee & Gillespie, 2007). Also, there is a possibility that soil possesses the ability to detoxify allelochemicals, so the bioassays conducted under laboratory conditions in the absence of soil might be misleading due to an overestimation of the allelopathic potential (Foy, 1999; Inderjit, 2001). Therefore, further work is required to study the allelopathic effect of *Brassica napus* on weeds species by residue incorporation or spraying the extract under field conditions.

The main limitation of this study was using Petri dishes to grow weeds inside enclosed plant growth chambers where some of the environmental factors are limited. The enclosed chamber led to a limitation of some abiotic factors such as different levels of water, air, humidity, soil, temperature and light, also, some biotic factors for example, plants, fungi, bacteria. The size of Petri dish and chambers also limited the number of weeds that could be grown, and as a consequence did not allow an investigation of all relevant parameters. Moreover, growing weeds in a Petri dish may lead to a restriction in weed growth and a possible limitation of nutrient availability to support weed growth while testing the effect of water extract for longer time.

Water extracts from all *B. napus* parts significantly affect all weed parameters and reduced seed germination, shoot length, root length, shoot fresh weight and root fresh weight. However, the degree of suppression effect of water extracts from different parts on weed parameters was varied. Our results were supported (Turk and Tawaha, 2003; Anjum *et al*., 2005; Toosi and Baki, 2012; Gella *et al*., 2013) as they report that extracts from different plant parts have different effect on weed species inhibition. This is assumed because of the variation in levels of glucosinolates in different plant parts as the results showed in chapters 4 and 6, where the concentrations and types of glucosinolates was varied between plant parts. There is evidence in the literature of enhanced increasing in the level of isothiocyanates released from glucosinolates after hydrolysis by myrosinase enzymes which have herbicidal properties (Norsworthy and Meehan 2005). Brown & Morra, (1996) suggested that enzymatic hydrolysis of glucosinolates in *Brassica spp* tissues releases a number of chemical compounds, mostly isothiocyanates, which will probably reduce seed germination. Several studies on *Brassica* showed that the isothiocyanates which are released from glucosinolates glucoiberin, glucoerucin, glucoraphanin, gluconapin, gluconasturtin, and glucotrapaeolin after

hydrolysis by myrosinase, have potential herbicidal properties and may also be used as biofumigants (Kirkegaard and Sarwar 1998). This suggestion supports our results in chapters 4 and 6, as the production of glucosinolates such as glucoberin, progoitrin, epi-progoitrin, gluconapin, glucobrassinapin, glucoraphanin, glucobrassicin, 4OH glucobrassicin, neoglucobrassicin and gluconasturtiin were observed through using HPLC analysis. Additionally, in chapter 5 the results showed that using pure glucosinolates in the laboratory with and without myrosinase can inhibit seed germination and seedling growth of weed species tested. Therefore, this finding provid evidence that glucosinolates contained in the *B. napus* tissues tested have a great effect on weed species germination and seedling growth. The inhibitory effect of the test extracts on seed germination and radicle length may be due to the presence of putative allelochemicals. The main constituents of brassica are several glucosinolates which are responsible for its wide ranging biological effects (Chandra et al., 2012). In the present study, allelopathic effect of brassica extract can be attributed to its glucosinolates content. The effect may be due to synergistic effect rather than single constituent.

From the this study, it can be concluded that *B. napus* parts exhibited remarkable negative allelopathic potential by significantly affecting the germination, shoot and root growth of *P. minor* was found to be more sensitive than *C. arvensis* and *S. halepense*. The observed allelopathic effect was plausibly due to its glucosinolates content. Further studies are necessary to determine the exact chemical constituents of B. napus accounting for its allelopathic activity. Allelopathic effects of *B. napus* under field conditions also need further research in pursuit of a new effective natural herbicide.

In the present study, water extract from flowers had the most effectiveness on seed germination and seedling growth of *Phalaris minor* and *Convolvulus arvensis,* and

no germination report in *Phalaris minor*, and also the germination reduced up to 93.88% and 90.5% for *Convolvulus arvensis* and *Sorghum halepense* respectively. Although the water extract from other parts was less effective on weed species germination and seedling growth, weed species tested parameters were inhibited significantly (Cheema *et al*., 2003; Turk and Tawaha, 2003). On the other hand, the stem extract had the greatest effect on *Sorghum halepense* seedling growth. The inhibition of weed species germination and seedling growth obtained with flowers extract was associated with the amount of toxic isothiocyanates released through enzymatic hydrolysis of glucosinolates, as the results in chapters 4 and 6 showed that the production of total glucosinolate in Brassica flower tissue was higher as compared with other parts (Baleroni *et al*., 2000; Peterson *et al*., 2001; Yasumoto *et al*., 2010; Walsh *et al*., 2014). In addition, the reduction in weed species seed germination and seedling growth of weed species has increased by increasing the concentration of water extract from all parts of *B. napus* (Tawaha and Turk, 2003; Jafariehyazdi and Javidfar, 2011; Al- Sherif *et al*., 2013). A further finding was that the inhibitory effect of water extract from different plant parts on weed species germination and seedling growth was increased with increasing the concentrations of the extracts, thus, increasing the isothiocyanates amount through enzymatic hydrolysis of glucosinolates (Bell and Muller, 1973; Brown & Morra, 1996; Baleroni *et al*., 2000; Yasumoto *et al*., 2010; Walsh *et al*., 2014; Bangarwa and Norsworthy, 2014). As a result, under high concentrations of water extracts from *B. napus*, seed germination can be completely inhibited because of deactivation of the hydrolytic enzymes taking part in seed germination. This inhibition in seed germination and seedling growth of all weed species agrees with germination (Turk *et al*., 2003) and growth (Turk *et al*., 2005) of alfalfa and radish. Moreover, these results are in line with the finding by (Ghareib *et al*., 2010; Hegab & Ghareib, 2010).

In this research, a plant development stage experiment was conducted to understand the link between times collecting samples from different parts of *B. napus* and glucosinolates concentration and myrosinase enzyme activity and their effect on weed species inhibition. This experiment showed a significant difference between plant parts of *B. napus* at different plant development stages in production of glucosinolates concentration and myrosinase enzyme activity, and their effect on weed species seed germination and seedling growth. Although all treatments significantly affect all the plant parameters, the results showed a variation in the effect of using water extract from different parts of *B. napus* on germination % and seedling growth of *Phalaris minor*, *Convolvulus arvensis* and *Sorghum halepense*. (Chon and Kim, 2002; Turk and Tawaha, 2002; Turk and Tawaha; 2003). Similar to the results obtained in chapters 2 and 3, using flower water extract at T7 and T8 completely inhibited the seed germination % of *Phalaris minor*. It was followed by stems extract from T1, T3 and T6 development stages as compared with the water extract from leaves and roots at same development stages. Additionally, flower extracts at T7 and T8 significantly affected *Convolvulus arvensis* and *Sorghum halepense* as the *Convolvulus arvensis* germination % was supressed up to 97.9% and 100% respectively and *Sorghum halepense* germination % was reduced up to 96.9% and 98.9% respectively. These results were in agreement with data observed by Jafariehyazdi and Javidfar (2011), who found that flowers and stems extract have a great effect on sunflower germination and seedling growth. They also documented that root length was more sensitive to the water extracts as compared with shoot length and this finding supports our results in chapters 2, 3, 4 and 6. Overall, these results may be due to an increase in the glucosinolates accumulation in the long photoperiod and under high temperatures during flowering stage, subsequently increasing in the amount of toxic isothiocyanates released through enzymatic hydrolysis of glucosinolates. Justen and Fritz (2013) reported that the glucosinolate

concentrations in *Brassica rapa* have been increased by raising the temperatures; also a positive relationship between glucosinolates and soil temperature has been found in *Brassica oleracea* (Charron and Sams, 2004). The present study demonstrated remarkable allelopathic potential of brassica against the weed seeds. The effect was possibly due to the glucosinolates contents of brassica. These results were obtained under laboratory conditions. The evaluation of the allelochemicals and their isolation, identification, release, and movement under field conditions are important guidelines for future research.

In the present study, the data showed that the effect of using water extract from same plant parts with same concentration on seed germination and seedling growth was slightly different in chapters 2, 3 and 4. This is probable because the plant used for extracts in chapter 2 was grown in the field, however for other experiments the *B. napus* used for extracts was grown in polytunnel under different conditions such as time of sowing, temperatures, day length, light, soil and humidity. Rice (1984) recorded that numerous factors may affect the allelochemicals produced by plants such as; temperature, light, water stress, mineral deficiency. Moreover, Mkula (2006) suggested that light is one of the factors that may affect the quantity of allelochemicals released by plants. Additionally, the increased concentration of allelochemicals in the presence of light might be because of the promotion effect of photosynthesis in chloroplasts (Cooner, 1987). Mølmann *et al*. (2015), reported a variation in individual glucosinolates with different temperatures and day length. Also, Steindal *et al*. (2015), documented that the content of individual glucosinolates in *B. oleracea* tissues are affected by temperature and photoperiod. Depending on glucosinolate type, the responses to the temperature and photoperiod was varied as they found that glucoiberin content reduces approximately up to 45% during a long day with high temperature (21/15 °C). Velasco *et al*. (2007) observed that the content of glucosinolate in *B. oleracea* plants was reduced when low growth

temperature reduced to freezing. However, the content of glucosinolate in leaves harvested in January, the coldest month, was slightly higher as compared with other treatments. It seems to be this is the main reason of the variation in concentration of individual glucosinolate and myrosinase activity and their content in different parts of *B. napus* under different harvesting time. Therefore, it was clear that the individual type and content of glucosinolate was affected by the time of harvesting samples from different *B napus* parts, sowing date and environment conditions.

In this research, seed germination and seedling growth were also influenced by applying pure glucosinolate; glucoraphanin, gluconasturtiin gluconapin and mixed glucosinolates with myrosinase enzyme and without enzyme at different concentrations. All glucosinolates at high concentration with myrosinase enzyme and without enzyme showed significant inhibition of seed germination (%) of weed species compared with other treatments. Glucoraphanin and gluconapin at 25µmol with myrosinase were the more effective on the germination of *Phalaris minor* and *Sorghum halepense*. However, the gluconasturtiin and glucoraphanin at 25 µmol with myrosinase were more effective than gluconapin when applied on *Convolvulus arvensis*. Shoot length, root length, shoot fresh weight and root fresh weight of all weed species were influenced when treated with high concentration of pure glucosinolates. It is clear that this finding supports our results obtained in chapters 2, 3, 4 and 6 by applying water from different parts of *B. napus* on weed species, which contain isothiocyanates released from glucosinolates through hydrolysis by myrosinase and have potential herbicidal effect (Brown & Morra, 1996; Kirkegaard and Sarwar, 1998; Baleroni *et al*., 2000; Yasumoto *et al*., 2010; Walsh *et al*., 2014; Bangarwa and Norsworthy, 2014). The most interesting observation in this experiment was the seed germination and seedling growth of all weed species was significantly reduced by all pure glucosinolates tested without myrosinase. However, the effect of myrosinase was often greater where higher concentrations of GSLs

were applied. These results are in contrast with previous findings by several researchers (Brown & Morra, 1996; Kirkegaard and Sarwar, 1998; Baleroni *et al*., 2000; Chon and Kim, 2002; Turk and Tawaha, 2002; Turk and Tawaha; 2003; Yasumoto *et al*., 2010; Walsh *et al*., 2014; Bangarwa and Norsworthy, 2014), as these authors report that isothiocyanates released from glucosinolates through hydrolysis by myrosinase and the myrosinase play a key role in the change of glucosinolate from nontoxic to toxic compound and have a potential herbicidal effect on germination and growth of plants. However, Gomaa *et al*. (2014), found that the osmotic potential of the aqueous extract from *Sonchus oleraceus* significantly lowered the total germination and the speed of accumulated germination of the target weed species. In addition, the osmotic potential of the plant extract at a concentration of 1% significantly reduced the root growth of *Melilotus indicus*, also the shoot growth of all target species except *Chenopodium murale*. Moreover, previous studies reported that both plant allelopathic and potential osmotic pressure of the aqueous extract may significantly inhibit seed germination and seedling growth (Wardle *et al*. 1992; Souza *et al*. 2010). Its main limitation was the unexamined the effect of pure GSL under natural conditions because of their little availability and the high cost.

Due to the significant effect of water stress on the synthesis of secondary plant products and accumulation of natural products in the *Brassica* plant tissue such as glucosinolates (Zhang *et al*., 2008; Taiz and Zeiger 2010; Selmar and Kleinwächter, 2013), an experiment was set up to investigate the effect of water stress levels during different plant growth stage on glucosinolates concentration and their effect on seed germination and seedling growth (chapter 6). Glucosinolate concentration, myrosinase activity, seed germination and seedling growth were significantly influenced by water stress. Ten individual glucosinolates were identified through High Performance Liquid Chromatography analysis (HPLC) analysis; the

concentration of progoitrin, glucoraphanin, gluconapin, gluconasturtiin and glucobrassinapin was the highest. Flowers, stems and leaves tissue contained the highest concentration of progoitrin, followed by glucobrassinapin under all treatments. However, the concentration of gluconasturtiin was the greatest in root tissue. Under WS2 and WS3, the glucosinolates concentration was slightly increased. A number of authors documented that glucosinolate biosynthesis in plants and their accumulation is affected by water stress conditions (Radovich *et al*., 2005; Robbins *et al*., 2005; Zhang *et al*., 2008). Additionally, numerous studies report that environmental stresses such as drought, salt stress, temperature and light significantly affect glucosinolates profile and concentration (Velasco *et al*., 2007; Yuan et al., 2010; Steindal *et al*., 2015). Furthermore, Endara and Coley (2011), have concluded that when plants are under stress their growth is frequently reduced more than photosynthesis, and carbon fixation is mostly invested to secondary metabolites production, and this may lead to an increase in the secondary metabolism and subsequently increase the glucosinolates. In agreement with this suggestion, Jones and Hartley (1999) suggested that plant growth parameters often reduce under water stress and secondary metabolites subsequently may increase at the expense of primary metabolism. Total glucosinolate is significantly higher in roots extract under all treatments followed by flowers extract, stems extract then leaf extract. Also, myrosinase activity was significantly higher in flower and leaf tissue, similar results were report in chapter 4. Several authors reported that the activity of myrosinase enzyme may depend on genetic variation, or may be due to ascorbic acid concentration, pH and temperature (Ludikhuyze *et al*., 2000; Rask *et al*., 2000). Hence, to achieve optimization in phytochemical content of *Brassica spp*. at each location, plant variety selection must be tailored to particular environmental factors in that location. Also, seed germination, shoot length, root length, shoot fresh weight and root fresh weight of

weed species tested significantly influenced by applying water extract from all parts under all water stress treatments. These results were strongly confirmed by the findings obtained in chapters 2, 3, 4 and 6 under control conditions where seed germination and seedling growth of all weed species tested in this project were reduced when treated with water extract from different parts from *B. napus*, under different concentrations and during different plant growth stages.

Laboratory bioassays are suitable for understanding different aspects of allelopathy such as release of allelochemicals from the donor plant, persistence in soil and uptake of allelochemicals but it is also important to know the fate of these allelochemicals in the soil and their interaction with abiotic and biotic influences. Therefore, further experimentation needs to be done to verify the validity of these observations in environments more closely resembling those occurring in nature. Moreover, the allelopathic effects exerted by plant extracts probably does not mirror well the natural release of allelochemicals from plants or their residual matter. In subsequent experiments it will be important to use soil as a growing medium, and that fresh plant material be used instead of the aqueous extract solutions.

In summary, the aims of this project have been examined through the studies presented in chapters 2 - 6 to investigate the possibilities of using *B. napus* water extracts for weed management. The results indicated that *B. napus* water extracts from different parts and at different levels significantly inhibit the seed germination and seedling growth of all weed species tested. Glucosinolate concentration and myrosinase activity varied in extracts prepared from *B. napus* samples harvested during different plant development stages and from different plant parts, also seed germination and seedling growth of all weed species tested were significantly suppressed when treated by water extract from those samples. Additionally, water extracts from *B. napus* under different water stress levels and plant development

stages show allelopathic activity against seed germination and seedling growth of all weed species tested. Also, progoitrin and glucobrassinapin concentration were the highest in frozen- dried flowers and stems tissues under all treatments. In the frozen-dried leaf tissues, progoitrin concentration was significantly higher. However, glucobrassinapin concentration was higher under WS2 during F and S+F plant development stages. At the same time, in frozen- dried root tissues the gluconasturtiin concentration significantly increased and recorded the highest value, followed by progoitrin then glucobrassinapin under all treatments. On the other hand, myrosinase activity increased significantly in flower and leaf extracts, followed by stem and root extracts, which recorded the lowest myrosinase activity. Flower extracts show no significant differences between the treatments on myrosinase activity. It is imperative to conclude whether these allelochemicals can accumulate under field conditions and effect of a weeds. This confirms the need to carry out field trials to quantify suppression caused by an allelopathic species. To determine allelopathic effects conclusively, the allelochemical has to be added in the environment and stay there long enough to be available for uptake by the target plant. In addition, the allelochemical must be detrimental to the target plant at typical concentrations and under realistic environmental conditions in order to play a significant ecological role (Choesin & Boerner, 1991).

In the present study, seed germination rate, shoot and root development were recorded to monitor the allelopathic action. Seed germination appeared to be the most sensitive parameter when treated by flower water extract, the results clearly indicated the allelopathic effect of *B. napus* extract on all tested weeds.

From these results, it is clear that *P. minor* was more sensitive to water extract from *B. napus* as compared to other species. Allelopathic effect was evaluated by recording the number of germinated seeds after 14 days. However, several

researchers evaluated the allelopathic effect on seed germination of weeds by recording the number of germinated seeds after 2, 3, 4 days (Turk and Tawaha, 2003; Chandra *at el.*, 2012) and 10 days (Nath *et al*., 2016) and that is mean the allelopathic effect from brassica parts have strong ability to prevent the germination of weeds. According to the outcomes of this project, the allelopathic phenomenon can be considered as a useful agricultural practice for weed management in field in order to reduce dependence on herbicides and achieve agroecosystem sustainability.

7.2. Recommendations for future studies

The allelopathic activity of *Brassica napus* extracts against weed species tested in this study may not act against other weed species. Future *in vitro* studies are necessary using *Brassica napus* water extracts with other weed species such as black-grass (*Alopecurus myosuroides*), barley grass (*Hordeum leporinum*), Bermuda grass (Cynodon dactylon) and common cocklebur (*Xanthium strumarium*) which have importance in farmer's fields. For GSL analysis, further work should investigate the use of different solvent for extractions such as hot water, methanol, chloroform, Petroleum ether and ethanol. In the study the results from *in vitro* experiments a showed that water extract affects weed species germination and seedling growth. However, this should be tested by residue incorporation or spraying the extract under field conditions. In this study, glucosinolate profiles and concentrations were determined. Further identification and quantifying of other chemical compounds in *B. napus* tissues are really required such as phenolic acids which may have allelopathic effect against weed species. Also, the effect of different development stages and water stress on total GSLs concentration were only investigated on one cultivar and others should be studied to determine if there is genotypic variance in these responses. The most effective water extract on weed

parameters was flower extract due to producing high levels of GSL associated with the release of toxic ITC. Extracts produced from flowers significantly inhibit the weed germination in the lab. Therefore, it would be of particular interest to investigate this effect under field conditions by incorporating the brassicas plant during flowering stage with soil or spraying the extract. Another necessary aspect to investigate is the combined effects of different allelopathic crop water extracts such as water extract from *B napus* with water extract from sunflower, rice, tobacco or sesame on weed species germination and seedling growth. In this study glucosinolate profiles and concentrations were determined. The activity of some allelochemicals may increase in soil. Further study is required to incorporate *B. napus* plant with soil during different plant growth stages.

7.3. Conclusions

From the overall investigation on the evaluation of allelopathic potential of brassica napus on weed species test, the following conclusions were drawn:

1- *Brassica napus* water extracts from the flowers appeared to be most effective treatment on seed germination and seedling growth of weed species, followed by stem extracts than leaf extracts.

2- Seed germination and seedling growth of all weed species tested in this study were significantly suppressed by high concentration of water extracts from all *B. napus* parts.

3- Seed germination of all three weed species were significantly inhibit by water extract from all *B. napus* parts collected at different plant development stages, water extract collected at T7 and T8 growth stages from all *B. napus* parts were the most effective, flower extract showed the most inhibitor effect on seed germination and seedling growth in comparison with other parts.

4- *Brassica napus* water extract showed allelopathic activity against root length of all weed species more than shoot length.

5- Total glucosinolates and myrosinase activity appeared to be highest in flower tissues collected at plant development stages T7 and T8, followed by root.

6- The levels of progoitrin and gluconasturtiin were the dominate glucosinolates in all tissues of *Brassica napus*.

7- Pure glucosinolates with myrosinase enzyme significantly affected the weed species as comparison with the pure glucosinolates without myrosinase enzyme. Glucoraphanin at 25 µmol showed the most inhibitor effect on *Convolvulus arvensis* and *Phalaris minor* germination and shoot length, however, gluconapin at 25 µmol was the most effective treatment on *Sorghum halepense* germination, shoot and root length.

8- Water stress levels during different plant growth stages influences glucosinolate concentrations and myrosinase activity and subsequently weed species parameters, however more study is required to understand the relationship between them.

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9. Appendices

Appendix 1. Kruskal-Wallis one-way analysis of variance and Kolmogorov-Smirnov two-sample test of weed species germinations %.

Kruskal-Wallis one-way analysis of variance of *Phalaris minor* (Retz.) seed germination%

Sample	Mean rank
Control	28.00
Whole plant water extract	19.70
Leaves water extract	12.00
Flowers water extract	3.00
Stems water extract	15.20
Root water extract	15.10
DF=5, Chi-square P<0.001, Value of H=22.11, Adjusted of ties =22.95	

Kolmogorov-Smirnov two-sample test of *Phalaris minor* (Retz.) seed germination %.

Difference between control & whole plant water extract	X_2=10,p=0.007
Difference between control & leaves water extract	X_2=10,p=0.007
Difference between control & flowers water extract	X_2=10,p=0.007
Difference between control & stems water extract	X_2=10,p=0.007
Difference between control & roots water extract	X_2=10,p=0.007

Difference between Whole plant & leaves water extract	X_2=3.6,p=0.167
Difference between whole plant & flowers water extract	X_2=10,p=0.007
Difference between whole plant & stems water extract	X_2=1.6,p=0.449
Difference between whole plant & roots water extract	X_2=3.6,p=0.1.65

Difference between leaves & flowers water extract	X_2=10,p=0.007
Difference between leaves & stems water extract	X_2=1.6,p=0.449
Difference between leaves & roots water extract	X_2=1.6,p=0.449

Difference between flowers & stems water extract	X_2=10,p=0.007
Difference between flowers & roots water extract	X_2=10,p=0.007

Difference between stems & roots water extract	X_2=0.4,p=0.819

Kruskal- Wallis one- way analysis of variance of *Convolvulus arvensis (*L.) Seed germination%.

Sample	Mean rank
Control	27.20
Whole plant water extract	19.80
Leaves water extract	11.90
Flowers water extract	3.00
Stems water extract	21.40
Root water extract	9.70
DF=5, Chi-square P<0.001, Value of H=25.36, Adjusted of ties=25.60	

Kolmogorov-Smirnov two-sample test of *Convolvulus arvensis* (L.) germination

Difference between control & whole plant water extract.	X_2=6.4,p=0.041
Difference between control & leaves water extract	X_2=10,p=0.007

Difference between control & flowers water extract	X_2=10,p=0.007
Difference between control & stems water extract	X_2=6.4,p=0.041
Difference between control & roots water extract	X_2=10,p=0.007

Difference between Whole plant & leaves water extract	X_2=3.6,p=0.165
Difference between whole plant & flowers water extract	X_2=10,p=0.007
Difference between whole plant & stems water extract	X_2=1.6,p=0.449
Difference between whole plant & roots water extract	X_2=6.4,p=0.041

Difference between leaves & flowers water extract	X_2=10,p=0.007
Difference between leaves & stems water extract	X_2=10,p=0.007
Difference between leaves & roots water extract	X_2=1.6,p=0.449

Difference between flowers & stems water extract	X_2=10,p=0.007
Difference between flowers & roots water extract	X_2=10,p=0.007

Difference between stems & roots water extract	X_2=10,p=0.007

Kruskal-Wallis one-way analysis of variance of *Sorghum halepense* (L.) seed germination %.

Sample	Mean rank
Control	27.60
Whole plant water extract	22.40
Leaves water extract	14.10
Flowers water extract	9.10
Stems water extract	7.30
Root water extract	12.50
DF=5, Chi-square P<0.001, Value of H=20.21, Adjusted of ties=20.84	

Kolmogorov-Smirnov two-sample test of *Sorghum halepense* (L.) seed germination%.

Difference between control & whole plant water extract.	X_2=6.4,p=0.041

Difference between control & leaves water extract	X_2=10,p=0.007
Difference between control & flowers water extract	X_2=10,p=0.007
Difference between control & stems water extract	X_2=10,p=0.007
Difference between control & roots water extract	X_2=10,p=0.007

Difference between Whole plant & leaves water extract	X_2=3.6,p=0.165
Difference between whole plant & flowers water extract	X_2=10,p=0.007
Difference between whole plant & stems water extract	X_2=10,p=0.007
Difference between whole plant & roots water extract	X_2=6.4,p=0.041

Difference between leaves & flowers water extract	X_2=1.6,p=0.449
Difference between leaves & stems water extract	X_2=3.6,p=0.165
Difference between leaves & roots water extract	X_2=1.6,p=0.449

Difference between flowers & stems water extract	X_2=1.6,p=0.449
Difference between flowers & roots water extract	X_2=3.6,p=0.165

Difference between stems & roots water extract	X_2=0.5,p=0.779

Appendix 2. Kruskal-Wallis one-way analysis of variance and Kolmogorov-Smirnov two-sample test of weed species shoot length (cm)

Kruskal-Wallis one-way analysis of variance of *Phalaris minor* (Retz.) shoots length (cm)

Sample	Mean rank
Control	28
Whole plant water extract	17.30
Leaves water extract	13.70
Flowers water extract	3.00
Stems water extract	14.80
Root water extract	16.2
DF=5, Chi-square P<0.001, Value of H=20.64, Adjusted of ties=20.75	

Kolmogorov-Smirnov two-sample test of *Phalaris minor (Retz.)* shoots length (cm)

Difference between control & whole plant water extract.	X_2=10,p=0.007
Difference between control & leaves water extract	X_2=10,p=0.007
Difference between control & flowers water extract	X_2=10,p=0.007
Difference between control & stems water extract	X_2=10,p=0.007
Difference between control & roots water extract	X_2=10,p=0.007

Difference between Whole plant & leaves water extract	X_2=1.6,p=0.449
Difference between whole plant & flowers water extract	X_2=10,p=0.007
Difference between whole plant & stems water extract	X_2=1.6,p=0.449
Difference between whole plant & roots water extract	X_2=1.6,p=0.449

Difference between leaves & flowers water extract	X_2=10 ,p=0.007
Difference between leaves & stems water extract	X_2=1.6,p=0.449
Difference between leaves & roots water extract	X_2=3.6,p=0.165

Difference between flowers & stems water extract	X_2=10,p=0.007
Difference between flowers & roots water extract	X_2=10,p=0.007

Difference between stems & roots water extract	X_2=1.6,p=0.449

Kruskal-Wallis one-way analysis of variance of *Convolvulus arvensis* (L.) shoots length

Sample	Mean rank
Control	26.60
Whole plant water extract	20.00
Leaves water extract	18.60
Flowers water extract	3.00
Stems water extract	13.60
Root water extract	14.00
DF=5, Chi-square P<0.001, Value of H=21.32, Adjusted of ties=21.34	

Kolmogorov-Smirnov two-sample test *Convolvulus arvensis* (L.) shoot length (cm)

Difference between control & whole plant water extract.	X_2=3.6,p=0.165
Difference between control & leaves water extract	X_2=10,p=0.007
Difference between control & flowers water extract	X_2=10,p=0.007
Difference between control & stems water extract	X_2=10,p=0.007
Difference between control & roots water extract	X_2=6.4,p=0.041

Difference between Whole plant & leaves water extract	X_2=6.4,p=0.41
Difference between whole plant & flowers water extract	X_2=10,p=0.007
Difference between whole plant & stems water extract	X_2=6.4,p=0.041
Difference between whole plant & roots water extract	X_2=3.6,p=0.165

Difference between leaves & flowers water extract	X_2=10,p=0.007
Difference between leaves & stems water extract	X_2=0.4,p=0.819
Difference between leaves & roots water extract	X_2=1.6,p=0.449

Difference between flowers & stems water extract	X_2=10,p=0.007
Difference between flowers & roots water extract	X_2=10,p=0.007

Difference between stems & roots water extract	X_2=3.6,p=0.165

Kruskal-Wallis one-way analysis of variance of *Sorghum halepense* (L.) shoots length (cm)

Sample	Mean rank

Control	28.00
Whole plant water extract	23.00
Leaves water extract	13.80
Flowers water extract	9.20
Stems water extract	6.60
Root water extract	12.40
DF=5, Chi-square P<0.001, Value of H=22.19, Adjusted of ties=20.38	

Kolmogorov-Smirnov two-sample test *Sorghum halepense* (L.) shoot length (cm)

Difference between control & whole plant water extract.	X_2=10,p=0.007
Difference between control & leaves water extract	X_2=10,p=0.007
Difference between control & flowers water extract	X_2=10,p=0.007
Difference between control & stems water extract	X_2=10,p=0.007
Difference between control & roots water extract	X_2=10,p=0.007

Difference between Whole plant & leaves water extract	X_2=10,p=0.007
Difference between whole plant & flowers water extract	X_2=10,p=0.007
Difference between whole plant & stems water extract	X_2=10,p=0.007
Difference between whole plant & roots water extract	X_2=10,p=0.007

Difference between leaves & flowers water extract	X_2=3.6,p=0.165
Difference between leaves & stems water extract	X_2=3.6,p=0.165
Difference between leaves & roots water extract	X_2=1.6,p=0.449

Difference between flowers & stems water extract	X_2=3.6,p=0.165
Difference between flowers & roots water extract	X_2=3.6,p=0.165

Difference between stems & roots water extract	X_2=3.6,p=0.165

Appendix 3. Kruskal-Wallis one-way analysis of variance and Kolmogorov-Smirnov two- sample test of weed species shoot weight (mg).

Kruskal-Wallis one-way analysis of variance of *Phalaris minor* (Retz.) shoots weight (g)

Sample	Mean rank
Control	28
Whole plant water extract	17
Leaves water extract	11.10
Flowers water extract	3.00
Stems water extract	16.60
Root water extract	17.30
DF=5, Chi-square P<0.001, Value of H=21.86, Adjusted of ties=20.97	

Kolmogorov-Smirnov two-sample test of *Phalaris minor* (Retz.) shoots weight (g)

Difference between control & whole plant water extract.	X_2=10,p=0.007
Difference between control & leaves water extract	X_2=10,p=0.007

Difference between control & flowers water extract	X_2=10,p=0.007
Difference between control & stems water extract	X_2=10,p=0.007
Difference between control & roots water extract	X_2=10,p=0.007

Difference between Whole plant & leaves water extract	X_2=3.6,p=0.165
Difference between whole plant & flowers water extract	X_2=10,p=0.007
Difference between whole plant & stems water extract	X_2=1.6,p=0.449
Difference between whole plant & roots water extract	X_2=1.6,p=0.449

Difference between leaves & flowers water extract	X_2=10,p=0.007
Difference between leaves & stems water extract	X_2=3.6,p=0.165
Difference between leaves & roots water extract	X_2=3.6,p=0.165

Difference between flowers & stems water extract	X_2=10,p=0.007
Difference between flowers & roots water extract	X_2=10,p=0.007

Difference between stems & roots water extract	X_2=0.4,p=0.819

Kruskal-Wallis one-way analysis of variance of *Convolvulus arvensis* (L.) shoots weight (g)

Sample	Mean rank
Control	28.00
Whole plant water extract	21.10
Leaves water extract	13.50
Flowers water extract	3.20
Stems water extract	14.50
Root water extract	12.70
DF=5, Chi-square P<0.001, Value of H=22.69, Adjusted of ties=22.75	

Kolmogorov-Smirnov two-sample test of *Convolvulus arvensis* (L.) shoots weight (g)

Difference between control & whole plant water extract.	X_2=10,p=0.007
Difference between control & leaves water extract	X_2=10,p=0.007
Difference between control & flowers water extract	X_2=10,p=0.007
Difference between control & stems water extract	X_2=10,p=0.007
Difference between control & roots water extract	X_2=10,p=0.007

Difference between Whole plant & leaves water extract	X_2=3.6,p=0.165
Difference between whole plant & flowers water extract	X_2=10,p=0.007
Difference between whole plant & stems water extract	X_2=3.6,p=0.165
Difference between whole plant & roots water extract	X_2=3.6,p=0.165

Difference between leaves & flowers water extract	X_2=10,p=0.007
Difference between leaves & stems water extract	X_2=1.6,p=0.449
Difference between leaves & roots water extract	X_2=2.69,p=0.261

Difference between flowers & stems water extract	X_2=10,p=0.007
Difference between flowers & roots water extract	X_2=4.5,p=0.105

Difference between stems & roots water extract	X_2=1.6,p=0.449

Kruskal-Wallis one-way analysis of variance of *Sorghum halepense* (L.) shoots weight (g)

Sample	Mean rank
Control	28,00
Whole plant water extract	21.50
Leaves water extract	12.80
Flowers water extract	11.90
Stems water extract	7.70
Root water extract	11.10
DF=5, Chi-square P<0.001, Value of H=18.88, Adjusted of ties=19.05	

Kolmogorov-Smirnov two-sample test of *Sorghum halepense* (L.) shoots weight (g)

Difference between control & whole plant water extract	X_2=10,p=0.007
Difference between control & leaves water extract	X_2=10,p=0.007
Difference between control & flowers water extract	X_2=10,p=0.007
Difference between control & stems water extract	X_2=10,p=0.007
Difference between control & roots water extract	X_2=10,p=0.007

Difference between Whole plant & leaves water extract	X_2=6.4,p=0.041
Difference between whole plant & flowers water extract	X_2=6.4,p=0.041
Difference between whole plant & stems water extract	X_2=6.4,p=0.041
Difference between whole plant & roots water extract	X_2=6.4,p=0.041

Difference between leaves & flowers water extract	X_2=0.4,p=0.819
Difference between leaves & stems water extract	X_2=3.6,p=0.165
Difference between leaves & roots water extract	X_2=1.6,p=0.449

Difference between flowers & stems water extract	X_2=3.6,p=0.165
Difference between flowers & roots water extract	X_2=1.6,p=0.449

Difference between stems & roots water extract	X_2=1.6,p=0.449

Appendix 4. Kruskal-Wallis one-way analysis of variance and Kolmogorov-Smirnov two-sample test of weed species root length (cm).

Kruskal-Wallis one-way analysis of variance of *Phalaris minor* (Retz.) root length (cm)

Sample	Mean rank
Control	26.60
Whole plant water extract	20.00
Leaves water extract	11.70
Flowers water extract	4.00
Stems water extract	19.00
Root water extract	11.70
DF=5, Chi-square P<0.001, Value of H=20.44, Adjusted of ties=20.71	

Kolmogorov-Smirnov two-sample test of *Phalaris minor* (Retz.) root length (cm)

Difference between control & whole plant water extract.	X_2=6.4,p=0.041

Difference between control & leaves water extract	X_2=6.4,p=0.041
Difference between control & flowers water extract	X_2=10,p=0.007
Difference between control & stems water extract	X_2=6.4,p=0.041
Difference between control & roots water extract	X_2=10,p=0.007

Difference between Whole plant & leaves water extract	X_2=6.4,p=0.041
Difference between whole plant & flowers water extract	X_2=10,p=0.007
Difference between whole plant & stems water extract	X_2=3.6,p=0.165
Difference between whole plant & roots water extract	X_2=6.4,p=0.041

Difference between leaves & flowers water extract	X_2=3.6,p=0.165
Difference between leaves & stems water extract	X_2=6.4,p=0.041
Difference between leaves & roots water extract	X_2=1.6,p=0.449

Difference between flowers & stems water extract	X_2=10,p=0.007
Difference between flowers & roots water extract	X_2=10,p=0.007

Difference between stems & roots water extract	X_2=6.4,p=0.014

Kruskal-Wallis one-way analysis of variance of *Convolvulus arvensis* (L.) root length (cm)

Sample	Mean rank
Control	28.00
Whole plant water extract	17.30
Leaves water extract	15.30
Flowers water extract	3.00
Stems water extract	16.30
Root water extract	13.10
DF=5, Chi-square P<0.001, Value of H=20.79, Adjusted of ties=20.98	

Kolmogorov - Smirnov two-sample test of *Convolvulus arvensis* (L.) root length (cm)

Difference between control & whole plant water extract.	X_2=10,p=0.007
Difference between control & leaves water extract	X_2=10,p=0.007
Difference between control & flowers water extract	X_2=10,p=0.007
Difference between control & stems water extract	X_2=10,p=0.007
Difference between control & roots water extract	X_2=10,p=0.007

Difference between Whole plant & leaves water extract	X_2=1.6,p=0.449
Difference between whole plant & flowers water extract	X_2=10,p=0.007
Difference between whole plant & stems water extract	X_2=04,p=0.819
Difference between whole plant & roots water extract	X_2=1.6,p=0.449

Difference between leaves & flowers water extract	X_2=10,p=0.007
Difference between leaves & stems water extract	X_2=0.4,p=0.819
Difference between leaves & roots water extract	X_2=1.6,p=0.449

Difference between flowers & stems water extract	X_2=10,p=0.007
Difference between flowers & roots water extract	X_2=10,p=0.007

Difference between stems & roots water extract	X_2=1.6,p=0.449

Kruskal-Wallis one-way analysis of variance of *Sorghum halepense* (L.) root length (cm)

Sample	Mean rank

Control	28.00
Whole plant water extract	13.80
Leaves water extract	11.50
Flowers water extract	11.50
Stems water extract	16.70
Root water extract	11.50
DF=5, Chi-square P<0.001, Value of H=13.46, Adjusted of ties=22.21	

Kolmogorov-Smirnov two-sample test of *Sorghum halepense* (L.) root length (cm)

Difference between control & whole plant water extract.	X_2=10,p=0.007
Difference between control & leaves water extract	X_2=10,p=0.007
Difference between control & flowers water extract	X_2=10,p=0.007
Difference between control & stems water extract	X_2=10,p=0.007
Difference between control & roots water extract	X_2=10,p=0.007

Difference between Whole plant & leaves water extract	X_2=0.4,p=0.819
Difference between whole plant & flowers water extract	X_2=0.4,p=0.819
Difference between whole plant & stems water extract	X_2=1.6,p=0.449
Difference between whole plant & roots water extract	X_2=0.4,p=0.819

Difference between leaves & flowers water extract	X_2=0.0,p=1
Difference between leaves & stems water extract	X_2=1.6,p=0.449
Difference between leaves & roots water extract	X_2=0.0,p=1

Difference between flowers & stems water extract	X_2=1.6,p=0.449
Difference between flowers & roots water extract	X_2=0.0,p=1

Difference between stems & roots water extract	X_2=1.6,p=0.449

Appendix 5. Kruskal-Wallis one-way analysis of variance and Kolmogorov-Smirnov two-sample test of weed species root weight (mg).

Kruskal-Wallis one-way analysis of variance of *Phalaris minor* (Retz.) root weight (mg)

Sample	Mean rank
Control	26.60
Whole plant water extract	17.60
Leaves water extract	8.90
Flowers water extract	4.00
Stems water extract	22.00
Root water extract	13.90
DF=5, Chi-square P<0.001, Value of H=22.47, Adjusted of ties=22.77	

Kolmogorov-Smirnov two-sample test of *Phalaris minor* (Retz.) root weight (mg)

Difference between control & whole plant water extract.	X_2=6.4,p=0.041
Difference between control & leaves water extract	X_2=10,p=0.007
Difference between control & flowers water extract	X_2=10,p=0.007
Difference between control & stems water extract	X_2=6.4,p=0.041
Difference between control & roots water extract	X_2=8.89,p=0.012

Difference between Whole plant & leaves water extract	X_2=6.4,p=0.041
Difference between whole plant & flowers water extract	X_2=10,p=0.007
Difference between whole plant & stems water extract	X_2=6.4,p=0.041
Difference between whole plant & roots water extract	X_2=6.4,p=0.041

Difference between leaves & flowers water extract	X_2=3.6,p=0.165
Difference between leaves & stems water extract	X_2=10 ,p=0.007
Difference between leaves & roots water extract	X_2=1.6,p=0.449

Difference between flowers & stems water extract	X_2=10,p=0.007
Difference between flowers & roots water extract	X_2=10,p=0.007

Difference between stems & roots water extract	X_2=6.4,p=0.0.041

Kruskal-Wallis one-way analysis of variance of *Convolvulus arvensis* (L.) root weight (mg)

Sample	Mean rank
Control	28.00
Whole plant water extract	21.10
Leaves water extract	13.50
Flowers water extract	3.20
Stems water extract	14.50
Root water extract	12.70
DF=5, Chi-square P<0.001, Value of H=22.69, Adjusted of ties=22.75	

Kolmogorov-Smirnov two-sample test of *Convolvulus arvensis* (L.) root weight (mg)

Difference between control & whole plant water extract.	X_2=10,p=0.007
Difference between control & leaves water extract	X_2=10,p=0.007
Difference between control & flowers water extract	X_2=10,p=0.007
Difference between control & stems water extract	X_2=10,p=0.007
Difference between control & roots water extract	X_2=10,p=0.007

Difference between Whole plant & leaves water extract	X_2=3.6,p=0.165
Difference between whole plant & flowers water extract	X_2=10,p=0.007
Difference between whole plant & stems water extract	X_2=3.6,p=0.165
Difference between whole plant & roots water extract	X_2=3.6,p=0.165

Difference between leaves & flowers water extract	X_2=10,p=0.007
Difference between leaves & stems water extract	X_2=1.6,p=0.449
Difference between leaves & roots water extract	X_2=1.6,p=0.449

Difference between flowers & stems water extract	X_2=10,p=0.007
Difference between flowers & roots water extract	X_2=6.4,p=0.041

Difference between stems & roots water extract	X_2=1.6,p=0.449

Kruskal-Wallis one-way analysis of variance of *Sorghum halepense* (L.) root weight (mg)

Sample	Mean rank
Control	28.00
Whole plant water extract	16.50
Leaves water extract	11.50
Flowers water extract	11.50
Stems water extract	14.00
Root water extract	11.50
DF=5, Chi-square P<0.001, Value of H=13.39, Adjusted of ties=22.09	

Kolmogorov-Smirnov two-sample test of *Sorghum halepense* (L.) root weight (mg)

Difference between control & whole plant water extract.	X_2=10,p=0.007
Difference between control & leaves water extract	X_2=10,p=0.007
Difference between control & flowers water extract	X_2=10,p=0.007
Difference between control & stems water extract	X_2=10,p=0.007
Difference between control & roots water extract	X_2=10,p=0.007

Difference between Whole plant & leaves water extract	X_2=1.6,p=0.449
Difference between whole plant & flowers water extract	X_2=1.6,p=0.449
Difference between whole plant & stems water extract	X_2=0.4,p=0.819
Difference between whole plant & roots water extract	X_2=1.6,p=0.449

Difference between leaves & flowers water extract	X_2=0.0,p=1
Difference between leaves & stems water extract	X_2=0.4,p=0.819
Difference between leaves & roots water extract	X_2=0.0,p=1

Difference between flowers & stems water extract	X_2=0.4,p=0.819
Difference between flowers & roots water extract	X_2=0.0,p=1

Difference between stems & roots water extract	X_2=0.4,p=0.819

Appendix 6. A Duncan's multiple range test to compare the differences between means of different factors used on *Phalaris minor*

Phalaris minor germination %

Duncan's multiple range test
Pure glucosinolates

	Mean	
Glucoraphanin	72.00	a
Gluconapin	73.25	b
Gluconasturtiin	77.33	c
Mixed GSL	80.75	d

Duncan's multiple range test
Concentration (µmol)

	Mean	
25	63.25	a
12.5	66.75	b

6.25	72.87	c
3.125	78.25	d
1.56	82.75	e
0	91.12	f

Duncan's multiple range test

Pure glucosinolates× Concentration (µmol)

	Mean	
Glucoraphanin 25	55.00	a
Gluconapin 25	57.50	a
Gluconapin 12.5	60.50	b
Glucoraphanin 12.5	62.00	b
Gluconapin 6.25	68.50	c
Glucoraphanin 6.25	69.00	c
Gluconasturtiin 25	69.50	c
Gluconasturtiin 12.5	71.00	cd
Mixed GSL 25	71.00	cd
Mixed GSL 12.5	73.50	de
Glucoraphanin 3.125	74.50	e
Gluconasturtiin 6.25	75.00	e
Gluconapin 3.125	78.00	f
Gluconasturtiin 3.125	78.50	f
Mixed GSL 6.25	79.00	f
Glucoraphanin 1.56	80.00	fg
Gluconasturtiin 1.56	80.50	fg
Mixed GSL 3.125	82.00	gh
Gluconapin 1.56	83.50	h
Mixed GSL 1.56	87.00	i
Gluconasturtiin 0	89.50	ij
Glucoraphanin 0	91.50	j
Gluconapin 0	91.50	j

Duncan's multiple range test

Pure glucosinolates × Myrosinase

	Mean	
Glucoraphanin +M	58.83	a
Gluconapin +M	63.50	b
Gluconasturtiin +M	69.50	c
Mixed GSL +M	76.50	d
Gluconapin -M	83.00	e
Mixed GSL -M	85.00	f
Gluconasturtiin -M	85.17	f
Glucoraphanin -M	85.17	f
Mixed GSL -M	92.00	j

Duncan's multiple range test

Concentration (µmol) ×Myrosinase

	Mean	
25 +M	46.50	a
12.5 +M	52.75	b
6.25 +M	62.50	c
3.125 +M	71.50	d
1.56 +M	78.50	e
25 -M	80.00	ef
12.5 -M	80.75	f
6.25 -M	83.25	g
3.125 -M	85.00	g
1.56 -M	87.00	h
0+M	90.75	i
0 -M	91.50	i

Duncan's multiple range test

Pure glucosinolates × Concentration (μmol) ×Myrosinase

	Mean	
Glucoraphanin 25+M	29.00	a
Gluconapin 25+M	37.00	b
Gluconapin 12.5 +M	41.00	c
Glucoraphanin 12.5 +M	43.00	c
Glucoraphanin 6.25 +M	55.00	d
Gluconapin 6.25 +M	55.00	d
Gluconasturtiin 25 +M	58.00	de
Gluconasturtiin 12.5 +M	60.00	ef
Mixed GSL 25 +M	62.00	efg
Glucoraphanin 3.125 +M	63.00	fgh
Gluconasturtiin 6.25 +M	65.00	gh
Mixed GSL 12.5 +M	67.00	h
Gluconasturtiin 3.125 +M	71.00	i
Glucoraphanin 1.56 +M	72.00	i
Gluconapin 3.125 +M	73.00	i
Gluconasturtiin 1.56 +M	75.00	ij
Mixed GSL 6.25 +M	75.00	ij
Gluconapin 25 -M	78.00	jk
Mixed GSL 3.125+M	79.00	jkl
Gluconapin 12.5 -M	80.00	klm
Mixed GSL 12.5 -M	80.00	klm
Mixed GSL 25 -M	80.00	klm
Glucoraphanin 12.5 -M	81.00	klmn
Glucoraphanin 25 -M	81.00	klmn
Gluconasturtiin 25 -M	81.00	klmn
Gluconasturtiin 12.5 -M	82.00	klmno
Gluconapin 6.25 -M	82.00	klmno
Glucoraphanin 6.25 -M	83.00	lmno
Gluconapin 1.56+M	83.00	lmno
Gluconapin 3.125 -M	83.00	lmno
Mixed GSL 6.25 -M	83.00	lmno
Gluconapin 1.56 -M	84.00	mnop
Mixed GSL 1.56+M	84.00	mnop
Gluconasturtiin 6.25 -M	85.00	nop
Mixed GSL 3.125 -M	85.00	nop
Gluconasturtiin 3.125 -M	86.00	opq
Glucoraphanin 3.125 -M	86.00	opq
Gluconasturtiin 1.56 -M	86.00	opq
Glucoraphanin 1.56 -M	88.00	pqr

Gluconasturtiin 0+M	88.00	pqr
Mixed GSL 1.56 -M	90.00	qr
Gluconasturtiin 0 -M	91.00	r
Glucoraphanin 0+M	91.00	r
Gluconapin 0 -M	91.00	r
Glucoraphanin 0 -M	92.00	r
Gluconapin 0+M	92.00	r
Mixed GSL 0 -M	92.00	r
Mixed GSL 0+M	92.00	r

Phalaris minor shoot length (cm)

Duncan's multiple range test
Pure glucosinolates

	Mean	
Glucoraphanin	6.208	a
Gluconapin	6.442	b
Gluconasturtiin	6.533	c
Mixed GSL	6.608	d

Duncan's multiple range test
Concentration (µmol)

	Mean	
25	5.642	a
12.5	5.895	b
6.25	6.307	c
3.125	6.560	d
1.56	6.840	e
0	7.442	f

Duncan's multiple range test
Pure glucosinolates × Concentration (µmol)

	Mean	
Glucoraphanin 25	5.020	a
Gluconapin 25	5.350	b
Glucoraphanin 12.5	5.590	c
Gluconapin 12.5	5.640	c
Mixed GSL 25	5.970	d
Glucoraphanin 6.25	6.140	de
Mixed GSL 12.5	6.140	de
Gluconasturtiin 12.5	6.210	ef
Gluconasturtiin 25	6.230	ef
Gluconasturtiin 6.25	6.290	efg

Gluconapin 6.25	6.350	fg
Glucoraphanin 3.125	6.390	fg
Mixed GSL 6.25	6.450	gh
Gluconasturtiin 3.125	6.470	gh
Mixed GSL 3.125	6.620	hi
Glucoraphanin 1.56	6.750	i
Gluconasturtiin 1.56	6.750	i
Gluconapin 3.125	6.760	i
Mixed GSL 1.56	6.790	i
Gluconapin 1.56	7.070	j
Gluconasturtiin 0	7.250	jk
Glucoraphanin 0	7.360	kl
Gluconapin 0	7.480	l
Mixed GSL 0	7.680	m

Duncan's multiple range test
Pure glucosinolates × Myrosinase

	Mean	
Glucoraphanin +M	5.390	a
Gluconapin +M	6.033	b
Gluconasturtiin +M	6.393	c
Mixed GSL +M	6.513	d
Gluconasturtiin -M	6.673	e
Mixed GSL -M	6.703	e
Gluconapin -M	6.850	f
Glucoraphanin -M	7.027	g

Duncan's multiple range test
Concentration (μmol) ×Myrosinase

Mean		
25 +M	4.810	a
12.5 +M	5.275	b
6.25 +M	6.010	c
3.125 +M	6.375	d
25 -M	6.475	de
12.5 -M	6.515	e
6.25 -M	6.605	ef
1.56 +M	6.700	fg
3.125 -M	6.745	g
1.56 -M	6.980	h
0 +M	7.325	i
0 -M	7.560	j

Duncan's multiple range test
Pure glucosinolates × Concentration (μmol) ×Myrosinase

	Mean	
Glucoraphanin 25 +M	3.220	a
Glucoraphanin 12.5 +M	4.320	b
Gluconapin 25 +M	4.340	b
Gluconapin 12.5 +M	4.760	c
Glucoraphanin 6.25 +M	5.520	d

Mixed GSL 25 +M	5.720	de
Glucoraphanin 3.125 +M	5.940	ef
Gluconasturtiin 25 +M	5.960	ef
Mixed GSL 12.5 +M	5.980	ef
Gluconapin 6.25 +M	6.020	fg
Gluconasturtiin 12.5 +M	6.040	fg
Gluconasturtiin 6.25 +M	6.200	fgh
Mix GSL 25 -M	6.220	fghi
Glucoraphanin 1.56+M	6.280	ghij
Mixed GSL 6.25+M	6.300	ghijk
Mixed GSL 12.5 -M	6.300	ghijk
Gluconapin 25 -M	6.360	hijk
Gluconasturtiin 6.25 -M	6.380	hijkl
Gluconasturtiin 12.5 -M	6.380	hijkl
Gluconasturtiin 3.125+M	6.420	hijklm
Gluconasturtiin 25 -M	6.500	hijklmn
Gluconasturtiin 3.125 -M	6.520	ijklmno
Gluconapin 12.5 -M	6.520	ijklmno
Mixed GSL 3.125+M	6.540	jklmnop
Gluconapin 3.125+M	6.600	klmnopq
Mixed GSL 6.25 -M	6.600	klmnopq
Gluconapin 6.25 -M	6.680	lmnopqr
Gluconasturtiin 1.56+M	6.680	lmnopqr
Mixed GSL 3.125 -M	6.700	mnopqr
Glucoraphanin 6.25 -M	6.760	nopqrs
Mixed GSL 1.56+M	6.780	nopqrst
Mixed GSL 1.56 -M	6.800	nopqrst
Glucoraphanin 25 -M	6.820	opqrst
Gluconasturtiin 1.56 -M	6.820	opqrst
Glucoraphanin 3.125 -M	6.840	pqrst
Glucoraphanin 12.5 -M	6.860	qrst

Gluconapin 3.125 -M	6.920	rst
Glucoraphanin 0+M	7.060	stu
Gluconapin 1.56+M	7.060	stu
Gluconasturtiin 0 +M	7.060	stu
Gluconapin 1.56 -M	7.080	tu
Glucoraphanin 1.56 -M	7.220	uv
Gluconapin 0+M	7.420	vw
Gluconasturtiin 0 -M	7.440	vw
Gluconapin 0 -M	7.540	wx
Mixed GSL 0 -M	7.600	wx
Glucoraphanin 0 -M	7.660	wx
Mixed GSL 0 +M	7.760	x

Phalaris minor root weight (mg)

Duncan's multiple range test
Pure glucosinolates

	Mean	
Mixed GSL	3.895	a
Gluconapin	4.048	b
Glucoraphanin	4.078	b
Gluconasturtiin	4.120	b

Duncan's multiple range test
Concentration (µmol)

	Mean	
25	3.462	a
12.5	3.637	b
6.25	3.827	c
3.125	4.042	d
1.56	4.312	e
0	4.930	f

Duncan's multiple range test
Pure glucosinolates × Concentration (µmol)

	Mean	
Mixed GSL 25	3.160	a
Mixed GSL 12.5	3.350	ab
Glucoraphanin 25	3.440	bc
Gluconapin 25	3.530	bcd
Mixed GSL 6.25	3.590	cd
Glucoraphanin 12.5	3.670	de
Gluconapin 12.5	3.680	de
Gluconasturtiin 25	3.720	def
Gluconapin 6.25	3.840	efg
Gluconasturtiin 12.5	3.850	efgh

Mixed GSL 3.125	3.900	fghi
Glucoraphanin 6.25	3.940	ghij
Gluconasturtiin 6.25	3.940	ghij
Gluconapin 3.125	4.060	hijk
Gluconasturtiin 3.125	4.090	ijk
Glucoraphanin 3.125	4.120	jk
Gluconapin 1.56	4.220	kl
Gluconasturtiin 1.56	4.270	kl
Glucoraphanin 1.56	4.370	l
Mixed GSL 1.56	4.390	l
Gluconasturtiin 0	4.850	m
Glucoraphanin 0	4.930	m
Gluconapin 0	4.960	m
Mixed GSL 0	4.980	m

Duncan's multiple range test
Pure glucosinolates ×Myrosinase

	Mean	
Mixed GSL M+	3.213	a
Glucoraphanin M+	3.633	b
Gluconapin M+	3.873	c
Gluconasturtiin M+	3.900	c
Gluconapin M-	4.223	d
Gluconasturtiin M-	4.340	e
Glucoraphanin M-	4.523	f
Mixed GSL M-	4.577	f

Duncan's multiple range test
Concentration (µmol) ×Myrosinase

	Mean	
25 M+	2.885	a
12.5 M+	3.090	b
6.25 M+	3.395	c
3.125 M+	3.695	d
1.56 M+	4.025	e
25 M-	4.040	e
12.5 M-	4.185	f
6.25 M-	4.260	fg
3.125 M-	4.390	g
1.56 M-	4.600	h
0M+	4.840	i
0 M-	5.020	j

Duncan's multiple range test
Pure glucosinolates × Concentration (µmol) × Myrosinase

	Mean	
Mix GSL 25 M+	2.160	a
Mix GSL 12.5 M+	2.420	ab
Glucoraphanin 25 M+	2.680	bc
Mix GSL 6.25 M+	2.780	cd
Glucoraphanin 12.5 M+	3.020	de

Mix GSL 3.125 M+	3.220	ef
Gluconapin 25 M+	3.280	efg
Gluconapin 12.5 M+	3.380	fgh
Gluconasturtiin 25 M+	3.420	fgh
Glucoraphanin 6.25 M+	3.500	fghi
Gluconasturtiin 12.5 M+	3.540	ghi
Gluconapin 6.25 M+	3.620	hij
Gluconasturtiin 6.25 M+	3.680	hijk
Glucoraphanin 3.125 M+	3.760	ijkl
Gluconapin 25 M-	3.780	ijkl
Mix GSL 1.56M+	3.880	jklm
Gluconapin 3.125M+	3.900	jklmn
Gluconasturtiin 3.125M+	3.900	jklmn
Glucoraphanin 1.56M+	3.980	klmno
Gluconapin 12.5 M-	3.980	klmno
Gluconasturtiin 25 M-	4.020	lmnop
Gluconapin 6.25 M-	4.060	lmnopq
Gluconasturtiin 1.56M+	4.120	mnopqr
Gluconapin 1.56M+	4.120	mnopqr
Gluconasturtiin 12.5 M-	4.160	mnopqrs
Mixed GSL 25 M-	4.160	mnopqrs
Glucoraphanin 25 M-	4.200	mnopqrs
Gluconasturtiin 6.25 M-	4.200	mnopqrs
Gluconapin 3.125 M-	4.220	nopqrs
Mixed GSL 12.5 M-	4.280	opqrst
Gluconasturtiin 3.125 M-	4.280	opqrst
Glucoraphanin 12.5 M-	4.320	pqrst
Gluconapin 1.56 M-	4.320	pqrst
Glucoraphanin 6.25 M-	4.380	qrst

Mixed GSL 6.25 M-	4.400	rst
Gluconasturtiin 1.56 M-	4.420	rst
Glucoraphanin 3.125 M-	4.480	stu
Mixed GSL 3.125 M-	4.580	tuv
Gluconasturtiin 0M+	4.740	uvw
Glucoraphanin 1.56 M-	4.760	uvw
Mixed GSL 0M+	4.820	vw
Glucoraphanin 0M+	4.860	vwx
Mixed GSL 1.56 M-	4.900	wx
Gluconapin 0M+	4.940	wx
Gluconasturtiin 0 M-	4.960	wx
Gluconapin 0 M-	4.980	wx
Glucoraphanin 0 M-	5.000	wx
Mixed GSL 0 M-	5.140	x

Phalaris minor **shoot weight (mg)**

Duncan's multiple range test
Pure glucosinolates

	Mean	
Glucoraphanin	6.793	a
Mixed GSL	7.278	b
Gluconasturtiin	7.438	c
Gluconapin	7.633	d

Duncan's multiple range test
Concentration (µmol)

	Mean	
25	6.330	a
12.5	6.707	b
6.25	6.975	c
3.125	7.372	d
1.56	7.887	e
0	8.442	f

Duncan's multiple range test
Pure glucosinolates ×Concentration (µmol)

	Mean	
Glucoraphanin 25	5.810	a
Glucoraphanin 12.5	6.120	b
Glucoraphanin 6.25	6.410	c
Mixed GSL 25	6.410	c
Gluconasturtiin 25	6.450	cd
Gluconapin 25	6.650	cde
Mixed GSL 12.5	6.700	de
Glucoraphanin 3.125	6.840	ef

Mixed GSL 6.25	6.850	ef
Gluconasturtiin 12.5	7.000	fg
Gluconapin 12.5	7.010	fg
Gluconasturtiin 6.25	7.220	gh
Mixed GSL 3.125	7.240	gh
Gluconapin 6.25	7.420	hi
Glucoraphanin 1.56	7.530	ij
Gluconasturtiin 3.125	7.650	ij
Mixed GSL 1.56	7.740	j
Gluconapin 3.125	7.760	j
Gluconasturtiin 1.56	8.010	k
Glucoraphanin 0	8.050	kl
Gluconapin 1.56	8.270	l
Gluconasturtiin 0	8.300	l
Gluconapin 0	8.690	m
Mixed GSL 0	8.730	m

Duncan's multiple range test
Pure glucosinolates × Myrosinase

	Mean	
Glucoraphanin +M	6.010	a
Mixed GSL +M	6.717	b
Gluconasturtiin +M	7.057	c
Gluconapin +M	7.120	c
Glucoraphanin -M	7.577	d
Gluconasturtiin -M	7.820	e
Mixed GSL -M	7.840	e
Gluconapin -M	8.147	f

Duncan's multiple range test
Concentration (μmol) ×Myrosinase

	Mean	
25% +M	5.325	a
12.5% +M	5.930	b
6.25% +M	6.290	c
3.125% +M	6.870	d
25% -M	7.335	e
12.5% -M	7.485	ef
1.56% +M	7.600	f
6.25% -M	7.660	f
3.125% -M	7.875	g
1.56% -M	8.175	h
0% +M	8.340	h
0% -M	8.545	i

Duncan's multiple range test
Pure glucosinolates × Concentration (μmol) ×Myrosinase

	Mean	
Glucoraphanin 25 +M	4.300	a
Glucoraphanin 12.5 +M	4.860	b
Glucoraphanin 6.25 +M	5.360	c
Gluconasturtiin 25 +M	5.580	cd
Mixed GSL 25 +M	5.640	cd

Gluconapin 25 +M	5.780	de
Mixed GSL 12.5 +M	6.040	ef
Mixed GSL 6.25 +M	6.060	ef
Glucoraphanin 3.125 +M	6.160	f
Gluconapin 12.5 +M	6.280	fg
Gluconasturtiin 12.5 +M	6.540	gh
Mixed GSL 3.125 +M	6.580	ghi
Gluconasturtiin 6.25 +M	6.820	hi
Gluconapin 6.25 +M	6.920	ij
Mixed GSL 25 -M	7.180	jk
Mixed GSL 1.56+M	7.260	jkl
Glucoraphanin 1.56+M	7.300	kl
Glucoraphanin 25 -M	7.320	kl
Gluconasturtiin 25 -M	7.320	kl
Gluconapin 3.125+M	7.320	kl
Mixed GSL 12.5 -M	7.360	klm
Glucoraphanin 12.5 -M	7.380	klm
Gluconasturtiin 3.125+M	7.420	klm
Gluconasturtiin 12.5 -M	7.460	klmn
Glucoraphanin 6.25 -M	7.460	klmn
Glucoraphanin 3.125 -M	7.520	klmno
Gluconapin 25 -M	7.520	klmno
Gluconasturtiin 6.25 -M	7.620	lmnop
Mixed GSL 6.25 -M	7.640	lmnopq
Gluconapin 12.5 -M	7.740	mnopqr
Glucoraphanin 1.56 -M	7.760	mnopqrs
Gluconasturtiin 1.56+M	7.840	nopqrst
Gluconasturtiin 3.125 -M	7.880	opqrst
Mixed GSL 3.125 -M	7.900	opqrst
Gluconapin 6.25 -M	7.920	pqrst
Gluconapin 1.56+M	8.000	pqrst

Glucoraphanin 0 -M	8.020	qrst
Glucoraphanin 0+M	8.080	rstu
Gluconasturtiin 0+M	8.140	stu
Gluconasturtiin 1.56 -M	8.180	tuv
Gluconapin 3.125 -M	8.200	tuv
Mixed GSL 1.56 -M	8.220	tuv
Gluconapin 0+M	8.420	uvw
Gluconasturtiin 0 -M	8.460	uvw
Gluconapin 1.56 -M	8.540	vw
Mixed GSL 0+M	8.720	wx
Mixed GSL 0 -M	8.740	wx
Gluconapin 0 -M	8.960 x	

Phalaris minor root weight (mg)

Duncan's multiple range test
Pure glucosinolates

	Mean	
Gluconapin	3.128	a
Mixed GSL	3.297	b
Gluconasturtiin	3.310	b
Glucoraphanin	3.443	c

Duncan's multiple range test
Concentration (µmol)

	Mean	
25	2.562	a
12.5	2.810	b
6.25	3.017	c
3.125	3.312	d
1.56	3.667	e
0	4.397	f

Duncan's multiple range test
Pure glucosinolates × Concentration (µmol)

	Mean	
Gluconapin 25	2.350	a
Gluconapin 12.5	2.560	ab
Gluconasturtiin 25	2.580	ab
Mixed GSL 25	2.640	bc
Glucoraphanin 25	2.680	bc
Gluconapin 6.25	2.730	bc
Gluconasturtiin 12.5	2.790	bc
Mixed GSL 12.5	2.850	cd
Glucoraphanin 12.5	3.040	de
Mixed GSL 6.25	3.040	de
Gluconasturtiin 6.25	3.050	de

Gluconapin 3.125	3.080	de
Glucoraphanin 6.25	3.250	ef
Mixed GSL 3.125	3.330	fg
Glucoraphanin 3.125	3.400	fg
Gluconasturtiin 3.125	3.440	fg
Mixed GSL 1.56	3.530	g
Gluconapin 1.56	3.550	g
Gluconasturtiin 1.56	3.790	h
Glucoraphanin 1.56	3.800	h
Gluconasturtiin 0	4.210	i
Mixed GSL 0	4.390	ij
Glucoraphanin 0	4.490	j
Gluconapin 0	4.500	j

Duncan's multiple range test
Pure glucosinolates ×Myrosinase

	Mean	
Mixed GSL M+	2.567	a
Gluconapin M+	2.623	a
Glucoraphanin M+	2.840	b
Gluconasturtiin M+	3.050	c
Gluconasturtiin M-	3.570	d
Gluconapin M-	3.633	d
Mixed GSL M-	4.027	e
Glucoraphanin M-	4.047	e

Duncan's multiple range test
Concentration (μmol) × Myrosinase

	Mean	
25 M+	1.845	a
12.5 M+	2.130	b
6.25 M+	2.435	c
3.125 M+	2.800	d
1.56 M+	3.205	e
25 M-	3.280	e
12.5 M-	3.490	f
6.25 M-	3.600	f
3.125M-	3.825	g
1.56 M-	4.130	h
0 M+	4.205	h
0 M-	4.590	i

Duncan's multiple range test
Pure glucosinolates × Concentration (μmol) × Myrosinase

	Mean	
Gluconapin 25M+	1.660	a
Glucoraphanin 25M+	1.720	a
Mixed GSL 25 M+	1.880	ab
Gluconapin 12.5M+	1.920	ab
Mixed GSL 12.5M+	2.000	ab

Gluconasturtiin 25M+	2.120	bc
Mixed GSL 6.25M+	2.200	bcd
Gluconapin 6.25M+	2.200	bcd
Glucoraphanin 12.5M+	2.220	bcd
Gluconasturtiin 12.5M+	2.380	cd
Mixed GSL 3.125M+	2.500	de
Glucoraphanin 6.25M+	2.520	de
Gluconapin 3.125M+	2.560	de
Mixed GSL 1.56M+	2.760	ef
Gluconasturtiin 6.25M+	2.820	ef
Glucoraphanin 3.125M+	2.860	efg
Gluconasturtiin 25 M-	3.040	fgh
Gluconapin 25M-	3.040	fgh
Gluconapin 1.56M+	3.100	fgh
Gluconasturtiin 12.5M-	3.200	gh
Gluconapin 12.5M-	3.200	gh
Gluconapin 6.25M-	3.260	hi
Gluconasturtiin 6.25M-	3.280	hij
Gluconasturtiin 3.125M-	3.280	hij
Glucoraphanin 1.56M+	3.320	hij
Mixed GSL 25M-	3.400	hijk
Gluconapin 3.125M-	3.600	ijkl
Gluconasturtiin 3.125M-	3.600	ijkl
Glucoraphanin 25M-	3.640	jklm
Gluconasturtiin 1.56M+	3.640	jklm
Mixed GSL 12.5M-	3.700	klmn
Glucoraphanin 12.5M-	3.860	lmno
Mixed GSL 6.25M-	3.880	lmno
Glucoraphanin 3.125M-	3.940	lmnop

Gluconasturtiin 1.56M-	3.940	lmnop
Glucoraphanin 6.25M-	3.980	mnop
Gluconapin 1.56M-	4.000	mnopq
Gluconasturtiin 0M+	4.060	nopqr
Mixed GSL 0M+	4.060	nopqr
Mixed GSL 3.125M-	4.160	opqr
Glucoraphanin 1.56M-	4.280	pqrs
Gluconapin 0M+	4.300	pqrs
Mixed GSL 1.56M-	4.300	pqrs
Gluconasturtiin 0M-	4.360	qrst
Glucoraphanin 0M+	4.400	rstu
Glucoraphanin 0M-	4.580	stu
Gluconapin 0M-	4.700	tu
Mixed GSL 0M-	4.720	u

Appendix 7. A Duncan's multiple range test to compare the differences between means of different factors used on Convolvulus arvensis

Duncan's multiple range test
Pure glucosinolates

Mean		
Glucoraphanin	69.67	a
Gluconapin	70.50	ab
Gluconasturtiin	71.50	b
Mixed GSL	74.83	c

Duncan's multiple range test
Concentration (μmol)

	Mean	
25	60.00	a
12.5	65.38	b

6.25	69.25	c
3.125	73.75	d
1.56	78.12	e
0	83.25	f

Duncan's multiple range test
Pure glucosinolates × Concentration (µmol)

	Mean	
Glucoraphanin 25	54.50	a
Gluconasturtiin 25	59.00	b
Gluconapin 25	60.50	bc
Gluconapin 12.5	63.00	bcd
Gluconasturtiin 12.5	63.50	cd
Glucoraphanin 12.5	64.50	cd
Gluconapin 6.25	66.00	de
Mixed GSL 25	66.00	de
Gluconasturtiin 6.25	69.00	ef
Glucoraphanin 6.25	70.00	ef
Mixed GSL 12.5	70.50	fg
Gluconapin 3.125	72.00	fgh
Mixed GSL 6.25	72.00	fgh
Glucoraphanin 3.125	72.50	fgh
Gluconasturtiin 3.125	74.50	ghi
Glucoraphanin 1.56	75.00	hij
Mixed GSL 3.125	76.00	hij
Gluconapin 1.56	78.00	ijk
Gluconasturtiin 1.56	79.00	jk
Mixed GSL 1.56	80.50	kl
Glucoraphanin 0	81.50	kl
Gluconapin 0	83.50	l
Gluconasturtiin 0	84.00	l
Mixed GSL 0	84.00	l

Duncan's multiple range test
Pure glucosinolates × Myrosinase

	Mean	
Glucoraphanin +M	64.17	a
Gluconasturtiin +M	64.50	a
Gluconapin +M	67.17	b
Mixed GSL +M	71.67	c
Gluconapin -M	73.83	cd
Glucoraphanin -M	75.17	d
Mixed GSL -M	78.00	e
Gluconasturtiin -M	78.50	e

Duncan's multiple range test
Concentration (µmol) × Myrosinase

Mean		
25 +M	50.00	a
12.5 +M	58.00	b
6.25 +M	64.25	c
25 -M	70.00	d
3.125 +M	70.50	d
12.5 -M	72.75	de
6.25 -M	74.25	ef
1.56 +M	76.00	f
3.125 -M	77.00	f
1.56 -M	80.25	g
0 +M	82.50	gh
0 -M	84.00	h

Duncan's multiple range test

Pure glucosinolates × Concentration (μmol) × Myrosinase

Mean		
Glucoraphanin 25 +M	39.00	a
Gluconasturtiin 25 +M	46.00	b
Gluconasturtiin 12.5 +M	52.00	c
Gluconapin 25+M	55.00	cd
Gluconapin 12.5 +M	57.00	cde
Glucoraphanin 12.5 +M	57.00	cde
Mixed GSL 25 +M	60.00	def
Gluconasturtiin 6.25 +M	61.00	def
Gluconapin 6.25 +M	62.00	ef
Glucoraphanin 6.25 +M	65.00	fg
Gluconapin 25 -M	66.00	fgh
Mix GSL 12.5 +M	66.00	fgh
Gluconapin 3.125 +M	69.00	ghi
Gluconapin 12.5 -M	69.00	ghi
Mixed GSL 6.25 +M	69.00	ghi
Glucoraphanin 3.125 +M	70.00	ghi
Glucoraphanin 25 -M	70.00	ghi
Gluconasturtiin 3.125 +M	70.00	ghi
Gluconapin 6.25 -M	70.00	ghi
Gluconasturtiin 25-M	72.00	hij
Mixed GSL 25 -M	72.00	hij
Glucoraphanin 12.5 -M	72.00	hij
Mixed GSL 3.125 +M	73.00	ijk
Glucoraphanin 1.56 +M	73.00	ijk
Glucoraphanin 3.125 -M	75.00	ijkl
Glucoraphanin 6.25 -M	75.00	ijkl
Gluconasturtiin 1.56 +M	75.00	ijkl
Gluconapin 3.125-M	75.00	ijkl
Mixed GSL 6.25 -M	75.00	ijkl
Mixed GSL 12.5 -M	75.00	ijkl

Gluconasturtiin 12.5 -M	75.00	ijkl
Gluconasturtiin 6.25 -M	77.00	jklm
Gluconapin 1.56+M	77.00	jklm
Glucoraphanin 1.56 -M	77.00	jklm
Gluconasturtiin 3.125 -M	79.00	klmn
Gluconapin 1.56 -M	79.00	klmn
Mixed GSL 1.56 +M	79.00	klmn
Mixed GSL 3.125 -M	79.00	klmn
Glucoraphanin 0 +M	81.00	lmn
Glucoraphanin 0 -M	82.00	mn
Mixed GSL 1.56 -M	82.00	mn
Gluconasturtiin 0 +M	83.00	mn
Gluconasturtiin 1.56 -M	83.00	mn
Gluconapin 0 +M	83.00	mn
Mixed GSL 0 +M	83.00	mn
Gluconapin 0 -M	84.00	n
Gluconasturtiin 0 -M	85.00	n
Mixed GSL 0 -M	85.00	n

Convolvulus arvensis shoot length(cm)

Duncan's multiple range test

Pure glucosinolates

	Mean	
Glucoraphanin	10.13	a
Mixed GSL	10.62	b
Gluconapin	10.65	b
Gluconasturtiin	10.66	b

Duncan's multiple range test

Concentration (µmol)

	Mean	
25	9.21	a
12.5	9.62	b
6.25	10.04	c
3.125	10.42	d
1.56	11.07	e
0	12.74	f

Duncan's multiple range test
Pure glucosinolates × Concentration (µmol)

	Mean	
Glucoraphanin 25	8.92	a
Gluconapin 25	9.25	ab
Gluconasturtiin 25	9.28	abc
Glucoraphanin 12.5	9.34	abc
Mixed GSL 25	9.41	bc
Glucoraphanin 6.25	9.65	bcd
Gluconasturtiin 12.5	9.66	bcd
Gluconapin 12.5	9.72	bcd
Mixed GSL 12.5	9.75	cd
Glucoraphanin 3.125	9.99	de
Gluconapin 6.25	10.05	def
Mixed GSL 6.25	10.06	def
Gluconasturtiin 6.25	10.38	efg
Glucoraphanin 1.56	10.41	efg
Mixed GSL 3.125	10.44	efg
Gluconapin 3.125	10.51	fg
Gluconasturtiin 3.125	10.74	g
Gluconasturtiin 1.56	11.25	h
Mixed GSL 1.56	11.29	h
Gluconapin 1.56	11.34	h
Glucoraphanin 0	12.50	i
Gluconasturtiin 0	12.63	ij
Mixed GSL 0	12.80	ij
Gluconapin 0	13.03	j

Duncan's multiple range test
Pure glucosinolates) × Myrosinase

	Mean	
Glucoraphanin +M	8.67	a
Mixed GSL +M	9.20	b
Gluconapin +M	9.41	b
Gluconasturtiin +M	9.41	b
Glucoraphanin -M	11.60	c
Gluconapin -M	11.89	d
Gluconasturtiin -M	11.90	d
Mixed GSL -M	12.05	d

Duncan's multiple range test
Concentration (µmol) × Myrosinase

	Mean	
25 +M	7.39	a
12.5 +M	7.86	b
6.25 +M	8.38	c
3.125 +M	8.90	d
1.56 +M	9.85	e
25 -M	11.03	f
12.5 -M	11.38	g
6.25 -M	11.68	h
3.125 -M	11.93	h
1.56 -M	12.29	i
0+M	12.64	j
0 -M	12.84	j

Duncan's multiple range test
Pure glucosinolates × Concentration (µmol) × Myrosinase

	Mean	
Glucoraphanin 25 +M	7.16	a
Gluconapin 25 +M	7.40	ab
Gluconasturtiin 25 +M	7.44	ab
Glucoraphanin 12.5 +M	7.52	abc
Mixed GSL 25 +M	7.58	abcd
Mixed GSL 12.5 +M	7.84	bcde
Glucoraphanin 6.25 +M	7.86	bcde
Gluconasturtiin 12.5 +M	7.98	bcde
Gluconapin 12.5 +M	8.08	bcdef
Mixed GSL 6.25 +M	8.20	cdefg
Glucoraphanin 3.125 +M	8.26	defg
Gluconapin 6.25 +M	8.46	efgh
Mixed GSL 3.125 +M	8.70	fghi
Glucoraphanin 1.56 +M	8.82	ghi
Gluconasturtiin 6.25 +M	9.02	hij
Gluconapin 3.125 +M	9.14	ij
Gluconasturtiin 3.125 +M	9.52	jk
Mixed GSL 1.56 +M	10.06	kl
Gluconasturtiin 1.56 +M	10.10	kl
Gluconapin 1.56 +M	10.42	l
Glucoraphanin 25 -M	10.68	lm
Gluconapin 25 -M	11.10	mn
Gluconasturtiin 25 -M	11.12	mn
Glucoraphanin 12.5 -M	11.16	mn
Mixed GSL 25 -M	11.24	mno
Gluconasturtiin 12.5 -M	11.34	mnop
Gluconapin 12.5 -M	11.36	mnop
Glucoraphanin 6.25 -M	11.44	nop
Gluconapin 6.25 -M	11.64	nopq
Mixed GSL 12.5 -M	11.66	nopq

Glucoraphanin 3.125 -M	11.72	nopqr
Gluconasturtiin 6.25 -M	11.74	nopqr
Gluconapin 3.125 -M	11.88	opqrs
Mixed GSL 6.25 -M	11.92	opqrst
Gluconasturtiin 3.125 -M	11.96	pqrst
Glucoraphanin 1.56 -M	12.00	pqrst
Mixed GSL 3.125 -M	12.18	qrstu
Gluconapin 1.56 -M	12.26	qrstuv
Glucoraphanin 0%+M	12.40	rstuv
Gluconasturtiin 1.56 -M	12.40	rstuv
Gluconasturtiin 0+M	12.42	rstuv
Mixed GSL 1.56 -M	12.52	stuvw
Glucoraphanin 0 -M	12.60	tuvw
Mixed GSL 0+M	12.80	uvw
Mixed GSL 0 -M	12.80	uvw
Gluconasturtiin 0 -M	12.84	uvw
Gluconapin 0+M	12.94	vw
Gluconapin 0-M	13.12	w

Convolvulus arvensis root length (cm)

Duncan's multiple range test

Pure glucosinolates

	Mean	
Glucoraphanin	6.668	a
Gluconasturtiin	7.025	b
Gluconapin	7.323	c
Mixed GSL	8.047	d

Duncan's multiple range test

Concentration (µmol)

	Mean	
25	5.937	a
12.5	6.195	b
6.25	6.690	c
3.125	7.252	d

1.56	8.005	e
0	9.515	f

Duncan's multiple range test
Pure glucosinolates × Concentration (μmol)

	Mean	
Gluconasturtiin 25	5.730	a
Glucoraphanin 25	5.800	ab
Gluconapin 25	5.890	abc
Glucoraphanin 12.5	5.900	abc
Gluconasturtiin 12.5	5.980	abcd
Glucoraphanin 6.25	6.110	bcde
Gluconapin 12.5	6.190	cdef
Glucoraphanin 3.125	6.300	defg
Mixed GSL 25	6.330	efg
Gluconasturtiin 6.25	6.490	fgh
Gluconapin 6.25	6.520	fgh
Glucoraphanin 1.56	6.640	gh
Mixed GSL 12.5	6.710	h
Gluconasturtiin 3.125	7.080	i
Gluconapin 3.125	7.110	i
Mixed GSL 6.25	7.640	j
Gluconasturtiin 1.56	7.790	j
Gluconapin 1.56	8.350	k
Mixed GSL 3.125	8.520	k
Gluconasturtiin 0	9.080	l
Mixed GSL 1.56	9.240	l
Glucoraphanin 0	9.260	l
Mixed GSL 0	9.840	m
Gluconapin 0	9.880	m

Duncan's multiple range test
Pure glucosinolates × Myrosinase

	Mean	
Glucoraphanin +M	4.480	a
Gluconapin +M	5.067	b
Gluconasturtiin +M	5.217	b
Mixed GSL +M	6.510	c
Gluconasturtiin -M	8.833	d
Glucoraphanin -M	8.857	d
Gluconapin -M	9.580	e
Mixed GSL -M	9.583	e

Duncan's multiple range test
Concentration (μmol) × Myrosinase

	Mean	
25 +M	2.930	a
12.5 +M	3.370	b
6.25 +M	4.220	c
3.125 +M	5.270	d
1.56 +M	6.650	e
25 -M	8.945	f
12.5 -M	9.020	fg
6.25 -M	9.160	fgh
3.125 -M	9.235	gh
1.56 -M	9.360	hi
0+M	9.470	i

0 -M	9.560	i

Duncan's multiple range test

Pure glucosinolates × Concentration (μmol) × Myrosinase

	Mean	
Gluconapin 25 +M	2.440	a
Gluconasturtiin 25 +M	2.880	b
Glucoraphanin 25 +M	2.980	bc
Gluconapin 12.5 +M	2.980	bc
Glucoraphanin 12.5 +M	3.160	bcd
Gluconasturtiin 12.5 +M	3.280	bcde
Mixed GSL 25 +M	3.420	cde
Glucoraphanin 6.25 +M	3.460	cde
Gluconapin 6.25 +M	3.500	de
Glucoraphanin 3.125 +M	3.740	ef
Mixed GSL 12.5 +M	4.060	fg
Gluconasturtiin 6.25 +M	4.200	gh
Glucoraphanin 1.56 +M	4.260	gh
Gluconapin 3.125 +M	4.620	h
Gluconasturtiin 3.125 +M	5.320	i
Mixed GSL 6.25 +M	5.720	i
Gluconasturtiin 1.56 +M	6.580	j
Gluconapin 1.56 +M	7.040	k
Mixed GSL 3.125 +M	7.400	k
Gluconasturtiin 25 -M	8.580	l
Glucoraphanin 25 -M	8.620	lm
Glucoraphanin 12.5 -M	8.640	lm
Gluconasturtiin 12.5 -M	8.680	lm
Mix GSL 1.56+M	8.720	lm
Glucoraphanin 6.25 -M	8.760	lmn
Gluconasturtiin 6.25 -M	8.780	lmno
Gluconasturtiin 3.125 -M	8.840	lmnop

Glucoraphanin 3.125 -M	8.860	lmnopq
Gluconasturtiin 1.56 -M	9.000	lmnopqr
Glucoraphanin 1.56 -M	9.020	lmnopqr
Gluconasturtiin 0+M	9.040	lmnopqrs
Gluconasturtiin 0 -M	9.120	mnopqrst
Glucoraphanin 0 -M	9.240	nopqrstu
Mixed GSL 25 -M	9.240	nopqrstuv
Glucoraphanin 0+M	9.280	opqrstuvw
Gluconapin 25 -M	9.340	pqrstuvwx
Mixed GSL 12.5 -M	9.360	qrstuvwx
Gluconapin 12.5 -M	9.400	rstuvwx
Gluconapin 6.25 -M	9.540	stuvwxy
Mixed GSL 6.25 -M	9.560	tuvwxy
Gluconapin 3.125 -M	9.600	tuvwxy
Mixed GSL 3.125 -M	9.640	uvwxy
Gluconapin 1.56 -M	9.660	uvwxy
Mixed GSL 0+M	9.740	uvwxy
Mixed GSL 1.56 -M	9.760	uwxy
Gluconapin 0+M	9.820	xy
Gluconapin 0 -M	9.940	y
Mixed GSL 0 -M	9.940	y

Convolvulus arvensis shoot weight

Duncan's multiple range test
Pure glucosinolates

	Mean	
Gluconasturtiin	100.3	a
Glucoraphanin	101.0	a
Gluconapin	101.4	a
Mixed GSL	104.6	b

Duncan's multiple range test
Concentration (µmol)

	Mean	
25%	89.0	a
12.5	93.0	b
6.25	96.0	c

3.125	100.8	d
1.56	107.1	e
0	125.1	f

Duncan's multiple range test
Pure glucosinolates × Concentration (µmol)

	Mean	
Mixed GSL 25	86.7	a
Gluconapin 25	89.5	ab
Gluconasturtiin 25	89.6	ab
Glucoraphanin 25	90.2	abc
Gluconasturtiin 12.5	92.4	bcd
Gluconapin 12.5	93.0	bcde
Mixed GSL 12.5	93.2	bcde
Glucoraphanin 12.5	93.3	bcde
Gluconapin 6.25	95.2	cdef
Gluconasturtiin 6.25	95.4	cdef
Glucoraphanin 6.25	95.7	def
Mixed GSL 6.25	97.9	efg
Glucoraphanin 3.125	98.2	efg
Gluconasturtiin 3.125	98.6	fg
Gluconapin 3.125	98.6	fg
Glucoraphanin 1.56	101.0	g
Gluconasturtiin 1.56	102.8	gh
Gluconapin 1.56	107.1	hi
Mixed GSL 3.125	107.6	i
Mixed GSL 1.56	117.4	j
Gluconasturtiin 0	123.1	k
Gluconapin 0	124.8	k
Mixed GSL 0	124.8	k
Glucoraphanin 0	127.6	k

Duncan's multiple range test
Pure glucosinolates × Myrosinase

	Mean	
Glucoraphanin + M	78.4	a
Gluconasturtiin + M	79.4	ab
Gluconapin + M	81.5	b
Mixed GSL + M	91.6	c
Mixed GSL - M	117.6	d
Gluconasturtiin - M	121.2	e
Gluconapin - M	121.3	e
Glucoraphanin - M	123.6	e

Duncan's multiple range test
Concentration (µmol) × Myrosinase

	Mean	
25 + M	61.8	a
12.5 + M	67.8	b
6.25 + M	72.5	c
3.125 + M	80.0	d
1.56 + M	90.3	e
25 - M	116.2	f
12.5 - M	118.1	f
6.25 - M	119.5	fg
3.125 - M	121.5	gh
1.56 - M	123.8	hi
0+ M	123.9	hi
0 - M	126.2	i

Duncan's multiple range test
Pure glucosinolates × Concentration (μmol) × Myrosinase

Mean		
Glucoraphanin 25 + M	60.8	a
Gluconasturtiin 25 + M	61.2	a
Gluconapin 25 + M	61.6	a
Mixed GSL 25 + M	63.4	ab
Glucoraphanin 12.5 + M	65.6	abc
Gluconasturtiin 12.5 + M	65.6	abc
Gluconapin 12.5 + M	67.4	abc
Glucoraphanin 6.25 + M	69.0	bcd
Gluconapin 6.25 + M	69.8	bcd
Gluconasturtiin 6.25 + M	70.4	bcd
Glucoraphanin 3.125 + M	72.4	cd
Mixed GSL 12.5 + M	72.8	cd
Gluconapin 3.125 + M	75.2	de
Gluconasturtiin 3.125 + M	75.6	de
Glucoraphanin 1.56 + M	76.0	def
Mixed GSL 6.25 + M	81.0	ef
Gluconasturtiin 1.56 + M	82.6	f
Gluconapin 1.56 + M	90.6	g
Mixed GSL 3.125 + M	96.8	g
Mixed GSL 25 - M	110.0	h
Mixed GSL 1.56+ M	112.0	hi
Mixed GSL 12.5 - M	113.6	hij
Mixed GSL 6.25 - M	114.8	hijk
Gluconapin 25 - M	117.4	ijkl
Gluconasturtiin 25 - M	118.0	ijklm
Mixed GSL 3.125 - M	118.4	ijklmn
Gluconapin 12.5 - M	118.6	ijklmn
Gluconasturtiin 12.5 - M	119.2	ijklmno
Glucoraphanin 25 - M	119.6	jklmno

Gluconasturtiin 6.25 - M	120.4	jklmno
Gluconapin 6.25 - M	120.6	jklmnop
Glucoraphanin 12.5 - M	121.0	jklmnop
Gluconasturtiin 0+ M	121.2	jklmnop
Gluconasturtiin 3.125 - M	121.6	klmnop
Gluconapin 3.125 - M	122.0	klmnop
Glucoraphanin 6.25 - M	122.4	klmnop
Mixed GSL 1.56 - M	122.8	lmnop
Gluconasturtiin 1.56 - M	123.0	lmnop
Gluconapin 1.56 - M	123.6	lmnop
Mixed GSL 0+ M	123.6	lmnop
Glucoraphanin 3.125 - M	124.0	lmnop
Gluconapin 0+ M	124.2	lmnop
Gluconasturtiin 0 - M	125.0	lmnop
Gluconapin 0 - M	125.4	mnop
Mixed GSL 0 - M	126.0	nop
Glucoraphanin 1.56 - M	126.0	mnop
Glucoraphanin 0+ M	126.8	op
Glucoraphanin 0 - M	128.4	p

Convolvulus arvensis root weight (mg)

Duncan's multiple range test

Pure glucosinolates

	Mean	
Glucoraphanin	16.70	a
Gluconasturtiin	17.77	b
Gluconapin	19.23	c
Mixed GSL	20.65	d

Duncan's multiple range test

Concentration (µmol)

	Mean	
25	15.24	a
12.5	16.18	b
6.25	17.36	c
3.125	18.88	d

1.56	20.29	e
0	23.57	f

Duncan's multiple range test
Pure glucosinolates × Concentration (µmol)

	Mean	
Glucoraphanin 25	13.68	a
Gluconasturtiin 25	14.38	ab
Glucoraphanin 12.5	14.55	abc
Gluconasturtiin 12.5	15.26	bcd
Glucoraphanin 6.25	15.40	bcd
Gluconapin 25	15.84	cde
Gluconasturtiin 6.25	16.53	def
Gluconapin 12.5	16.92	efg
Glucoraphanin 3.125	17.03	efgh
Mixed GSL 25	17.06	efgh
Gluconapin 6.25	17.53	fghi
Gluconasturtiin 3.125	17.98	ghi
Mixed GSL 12.5	18.00	ghi
Glucoraphanin 1.56	18.45	hi
Gluconasturtiin 1.56	18.65	i
Gluconapin 3.125	18.67	i
Mixed GSL 6.25	19.98	j
Glucoraphanin 0	21.10	jk
Gluconapin 1.56	21.25	jk
Mixed GSL 3.125	21.85	kl
Mixed GSL 1.56	22.80	lm
Gluconasturtiin 0	23.80	mn
Mix GSL 0	24.20	no
Gluconapin 0	25.20	o

Duncan's multiple range test
Pure glucosinolates × Myrosinase

	Mean	
Gluconapin +M	13.87	a
Glucoraphanin +M	13.88	a
Gluconasturtiin +M	13.97	a
Mixed GSL +M	17.87	b
Glucoraphanin -M	19.52	c
Gluconasturtiin -M	21.57	d
Mixed GSL -M	23.43	e
Gluconapin -M	24.60	f

Duncan's multiple range test
Concentration (µmol) × Myrosinase

	Mean	
25 +M	9.39	a
12.5 +M	10.90	b
6.25 +M	12.77	c
3.125 +M	15.37	d
1.56 +M	17.58	e
25 -M	21.08	f
12.5 -M	21.46	fg
6.25 -M	21.95	fg
3.125 -M	22.39	gh
1.56 -M	22.99	hi
0+M	23.35	i

0 -M	23.80	i

Duncan's multiple range test

Pure glucosinolates × Concentration (μmol) × Myrosinase

	Mean	
Gluconapin 25 +M	8.18	a
Gluconasturtiin 25 +M	8.60	ab
Glucoraphanin 25 +M	9.20	abc
Gluconasturtiin 12.5 +M	10.00	abcd
Gluconapin 12.5 +M	10.00	abcd
Glucoraphanin 12.5 +M	10.40	bcd
Gluconapin 6.25 +M	10.80	cde
Mixed GSL 25 +M	11.60	def
Glucoraphanin 6.25 +M	11.70	def
Gluconasturtiin 6.25 +M	11.80	def
Gluconapin 3.125 +M	12.62	efg
Mixed GSL 12.5 +M	13.20	fgh
Gluconasturtiin 3.125 +M	14.26	gh
Glucoraphanin 3.125 +M	14.40	gh
Gluconasturtiin 1.56 +M	14.94	hi
Glucoraphanin 1.56 +M	16.60	ij
Mixed GSL 6.25 +M	16.80	ijk
Gluconapin 1.56 +M	17.20	jkl
Glucoraphanin 25 -M	18.16	jklm
Glucoraphanin 12.5 -M	18.70	klmn
Glucoraphanin 6.25 -M	19.10	lmno
Glucoraphanin 3.125 -M	19.66	mnop
Gluconasturtiin 25 -M	20.16	nop
Mixed GSL 3.125+M	20.20	nop
Glucoraphanin 1.56 -M	20.30	nopq
Gluconasturtiin 12.5 -M	20.52	nopqr
Glucoraphanin 0+M	21.00	opqrs

Glucoraphanin 0 -M	21.20	pqrst
Gluconasturtiin 6.25 -M	21.26	pqrst
Mixed GSL 1.56+M	21.60	pqrstu
Gluconasturtiin 3.125 -M	21.70	pqrstu
Gluconasturtiin 1.56 -M	22.36	qrstuv
Mixed GSL 25 -M	22.52	rstuvw
Mixed GSL 12.5 -M	22.80	stuvwx
Mixed GSL 6.25 -M	23.16	tuvwxy
Gluconasturtiin 0 -M	23.40	uvwxy
Gluconapin 25 -M	23.50	uvwxy
Mixed GSL 3.125 -M	23.50	uvwxy
Mixed GSL 0+M	23.80	vwxy
Gluconapin 12.5 -M	23.84	vwxy
Mixed GSL 1.56 -M	24.00	vwxyz
Gluconasturtiin 0+M	24.20	vwxyz
Gluconapin 6.25 -M	24.26	vwxyz
Gluconapin 0+M	24.40	vwxyz
Mixed GSL 0 -M	24.60	wxyz
Gluconapin 3.125 -M	24.72	xyz
Gluconapin 1.56 -M	25.30	yz
Gluconapin 0 -M	26.00	z

Appendix 8. A Duncan's multiple range test to compare the differences between means of different factors used on Sorghum halepense

***Sorghum halepense* germination %**

Duncan's multiple range test

Pure glucosinolates

	Mean	
Glucoraphanin	65.83	a
Gluconapin	66.83	b
Gluconasturtiin	67.83	c
Mixed GSL	72.92	d

Duncan's multiple range test

Concentration (µmol)

	Mean	
25	57.88	a
12.5	63.00	b
6.25	67.38	c
3.125	70.38	d
1.56	73.25	e
0	78.25	f

Duncan's multiple range test
Pure glucosinolates × Concentration (µmol)

	Mean	
Glucoraphanin 25	49.50	a
Gluconapin 25	55.50	b
Gluconasturtiin 25	58.50	c
Glucoraphanin 12.5	60.00	cd
Gluconapin 12.5	61.00	d
Gluconasturtiin 12.5	62.00	d
Gluconapin 6.25	65.50	e
Gluconasturtiin 6.25	66.00	ef
Glucoraphanin 6.25	66.50	efg
Mixed GSL 25	68.00	fgh
Gluconapin 3.125	68.50	gh
Mixed GSL 12.5	69.00	h
Glucoraphanin 3.125	70.00	hi
Gluconasturtiin 3.125	70.00	hi
Mixed GSL 6.25	71.50	ij
Glucoraphanin 1.56	72.50	j
Gluconapin 1.56	72.50	j
Gluconasturtiin 1.56	73.00	jk
Mixed GSL 3.125	73.00	jk
Mixed GSL 1.56	75.00	kl
Glucoraphanin 0	76.50	lm
Gluconasturtiin 0	77.50	m
Gluconapin 0	78.00	m
Mixed GSL 0	81.00	n

Duncan's multiple range test
Pure glucosinolates × Concentration (µmol)

	Mean	
Glucoraphanin +M	59.00	a
Gluconapin +M	59.83	a
Gluconasturtiin +M	62.17	b
Mixed GSL +M	71.83	c
Glucoraphanin -M	72.67	cd
Gluconasturtiin -M	73.50	de
Gluconapin -M	73.83	de
Mixed GSL -M	74.00	e

Duncan's multiple range test
Concentration (µmol) × Myrosinase

	Mean	
25 +M	46.00	a
12.5 +M	55.25	b
6.25 +M	61.50	c
3.125 +M	66.25	d
25 -M	69.75	e
12.5 -M	70.75	ef
1.56+M	71.75	f
6.25 -M	73.25	g
3.125 -M	74.50	g
1.56 -M	74.75	g
0 -M	78.00	h

0+M	78.50	h

Duncan's multiple range test
Pure glucosinolates x Concentration (µmol) × Myrosinase

	Mean	
Glucoraphanin 25 +M	30.00	a
Gluconapin 25 +M	41.00	b
Gluconasturtiin 25 +M	47.00	c
Glucoraphanin 12.5 +M	50.00	d
Gluconasturtiin 12.5 +M	52.00	d
Gluconapin 12.5 +M	52.00	d
Gluconapin 6.25 +M	56.00	e
Glucoraphanin 6.25 +M	60.00	f
Gluconasturtiin 6.25 +M	60.00	f
Gluconapin 3.125 +M	62.00	f
Gluconasturtiin 3.125 +M	66.00	g
Glucoraphanin 3.125 +M	66.00	g
Mixed GSL 25 +M	66.00	g
Mixed GSL 12.5 +M	67.00	gh
Glucoraphanin 25 -M	69.00	ghi
Gluconapin 1.56+M	70.00	hij
Gluconasturtiin 25 -M	70.00	hij
Mixed GSL 6.25+M	70.00	hij
Glucoraphanin 12.5 -M	70.00	hij
Gluconapin 12.5 -M	70.00	hij
Gluconapin 25 -M	70.00	hij
Mixed GSL 25 -M	70.00	hij
Glucoraphanin 1.56+M	71.00	ijk
Gluconasturtiin 1.56+M	71.00	ijk
Mixed GSL 3.125+M	71.00	ijk
Mixed GSL 12.5 -M	71.00	ijk
Gluconasturtiin 6.25 -M	72.00	ijkl
Gluconasturtiin 12.5 -M	72.00	ijkl

Glucoraphanin 6.25 -M	73.00	jklm
Mixed GSL 6.25 -M	73.00	jklm
Glucoraphanin 1.56 -M	74.00	klmn
Glucoraphanin 3.125 -M	74.00	klmn
Gluconasturtiin 3.125 -M	74.00	klmn
Gluconasturtiin 1.56 -M	75.00	lmno
Gluconapin 1.56 -M	75.00	lmno
Gluconapin 3.125 -M	75.00	lmno
Gluconapin 6.25 -M	75.00	lmno
Mixed GSL 1.56 -M	75.00	lmno
Mixed GSL 1.56+M	75.00	lmno
Mixed GSL 3.125 -M	75.00	lmno
Glucoraphanin 0 -M	76.00	mno
Glucoraphanin 0+M	77.00	nop
Gluconasturtiin 0+M	77.00	nop
Gluconapin 0+M	78.00	op
Gluconasturtiin 0 -M	78.00	op
Gluconapin 0 -M	78.00	op
Mixed GSL 0 -M	80.00	pq
Mixed GSL 0+M	82.00	q

Sorghum halepense shoot length (cm)

Duncan's multiple range test
Pure glucosinolates

	Mean	
gluconapin	13.82	a
Gluconasturtiin	14.29	b
Mixed GSL	14.85	c
Glucoraphanin	15.13	d

Duncan's multiple range test
Concentration (μmol)

	Mean	
25	12.70	a
12.5	13.44	b
6.25	14.31	c
3.125	14.85	d
1.56	15.33	e

0	16.51	f

Duncan's multiple range test
Pure glucosinolates x Concentration (µmol)

	Mean	
Gluconasturtiin 25	12.13	a
gluconapin 25	12.24	a
Gluconasturtiin 12.5	12.76	b
gluconapin 12.5	12.77	b
Glucoraphanin 25	12.95	b
gluconapin 6.25	13.32	c
Mixed GSL 25	13.49	cd
Mixed GSL 12.5	13.68	cd
gluconapin 3.125	13.85	d
gluconapin 1.56	14.20	e
Gluconasturtiin 6.25	14.27	e
Mixed GSL 6.25	14.50	ef
Glucoraphanin 12.5	14.55	ef
Gluconasturtiin 3.125	14.76	f
Glucoraphanin 6.25	15.14	g
Mixed GSL 3.125	15.24	gh
Gluconasturtiin 1.56	15.48	ghi
Glucoraphanin 3.125	15.53	hi
Mixed GSL 1.56	15.71	ij
Glucoraphanin 1.56	15.93	j
Gluconasturtiin 0	16.35	k
Mixed GSL 0	16.48	k
gluconapin 0	16.54	k
Glucoraphanin 0	16.67	k

Duncan's multiple range test
Pure glucosinolates x Myrosinase

	Mean	
gluconapin +M	11.95	a
Gluconasturtiin +M	12.96	b
Mixed GSL +M	14.20	c
Glucoraphanin +M	14.44	d
Mixed GSL -M	15.50	e
Gluconasturtiin -M	15.63	ef
gluconapin -M	15.69	ef
Glucoraphanin -M	15.82	f

Duncan's multiple range test
Concentration (µmol) × Myrosinase

	Mean	
25 +M	10.43	a
12.5 +M	11.72	b
6.25 +M	13.19	c
3.125 +M	14.00	d
1.56 +M	14.61	e
25 -M	14.98	f
12.5 -M	15.17	f
6.25 -M	15.43	g
3.125 -M	15.69	h
1.56 -M	16.05	i
0+M	16.38	j
0 -M	16.65	k

Duncan's multiple range test
Pure glucosinolates x Concentration (µmol) × Myrosinase

	Mean	
gluconapin 25 +M	9.42	a
Gluconasturtiin 25 +M	9.46	a
gluconapin 12.5 +M	10.24	b
Gluconasturtiin 12.5 +M	10.44	b
Glucoraphanin 25 +M	10.64	bc
gluconapin 6.25 +M	11.10	c
gluconapin 3.125 +M	12.12	d
Mixed GSL 25 +M	12.18	d
gluconapin 1.56 +M	12.40	d
Mixed GSL 12.5 +M	12.48	d
Gluconasturtiin 6.25 +M	13.22	e
Glucoraphanin 12.5 +M	13.70	ef
Mixed GSL 6.25 +M	13.78	f
Gluconasturtiin 3.125 +M	13.84	f
Glucoraphanin 6.25 +M	14.66	g
Mixed GSL 3.125 +M	14.80	gh
Gluconasturtiin 25 -M	14.80	gh
Mixed GSL 25 -M	14.80	gh
Gluconasturtiin 1.56%+M	14.84	ghi
Mixed GSL 12.5 -M	14.88	ghi
gluconapin 25 -M	15.06	ghij
Gluconasturtiin 12.5 -M	15.08	ghij
Mixed GSL 6.25 -M	15.22	ghijk
Glucoraphanin 3.125+M	15.24	ghijkl
Glucoraphanin 25 -M	15.26	hijkl
gluconapin 12.5 -M	15.30	hijkl
Gluconasturtiin 6.25 -M	15.32	hijkl
Glucoraphanin 12.5 -M	15.40	ijklm
Mixed GSL 1.56+M	15.42	ijklmn
gluconapin 6.25 -M	15.54	jklmno
gluconapin 3.125 -M	15.58	jklmno

Glucoraphanin 6.25 -M	15.62	jklmno
Mixed GSL 3.125 -M	15.68	klmno
Gluconasturtiin 3.125 -M	15.68	klmno
Glucoraphanin 1.56+M	15.78	klmno
Glucoraphanin 3.125 -M	15.82	lmno
Gluconasturtiin 0+M	15.94	mnop
gluconapin 1.56 -M	16.00	nopq
Mixed GSL 1.56 -M	16.00	nopq
Glucoraphanin 1.56 -M	16.08	opqr
Gluconasturtiin 1.56 -M	16.12	opqr
Mixed GSL 0 -M	16.44	pqrs
gluconapin 0+M	16.44	pqrs
Mixed GSL 0+M	16.52	qrs
Glucoraphanin 0+M	16.60	rs
gluconapin 0 -M	16.64	rs
Glucoraphanin 0 -M	16.74	s
Gluconasturtiin 0-M	16.76	s

Sorghum halepense root length (cm)

Duncan's multiple range test
Pure glucosinolates

	Mean	
Gluconapin	8.978	a
Gluconasturtiin	9.062	a
Glucoraphanin	9.088	a
Mixed GSL	9.110	a

Duncan's multiple range test
Concentration (µmol)

	Mean	
25	7.620	a
12.5	7.887	b
6.25	8.195	c
3.125	8.500	d
1.56	8.765	e
0	13.390	f

Duncan's multiple range test
Pure glucosinolates x Concentration (µmol)

	Mean	
Gluconapin 25	7.530	a
Glucoraphanin 25	7.590	a
Gluconasturtiin 25	7.620	ab

Gluconapin 12.5	7.710	ab
Mixed GSL 25	7.740	ab
Gluconasturtiin 12.5	7.880	abc
Glucoraphanin 12.5	7.970	bcd
Mixed GSL 12.5	7.990	bcd
Gluconapin 6.2	8.120	cde
Mixed GSL 6.25	8.170	cdef
Glucoraphanin 6.25	8.220	cdefg
Gluconasturtiin 6.25	8.270	defg
Gluconapin 3.125	8.410	efgh
Gluconasturtiin 3.125	8.510	fgh
Glucoraphanin 3.125	8.520	fgh
Mixed GSL 3.125	8.560	ghi
Gluconapin 1.56	8.660	hi
Gluconasturtiin 1.56	8.720	hi
Mixed GSL 1.56	8.760	hi
Glucoraphanin 1.56	8.920	i
Glucoraphanin 0	13.310	j
Gluconasturtiin 0	13.370	j
Mixed GSL	13.440	j
Gluconapin 0	13.440	j

Duncan's multiple range test
Pure glucosinolates) × Myrosinase

	Mean	
Gluconapin +M	4.670	a
Gluconasturtiin +M	4.877	b
Mixed GSL +M	4.880	b
Glucoraphanin +M	5.123	c
Glucoraphanin -M	13.053	d
Gluconasturtiin -M	13.247	e
Gluconapin -M	13.287	e
Mixed GSL -M	13.340	e

Duncan's multiple range test
Concentration (μmol) × Myrosinase

	Mean	
25 +M	2.335	a
12.5 +M	2.720	b
6.25 +M	3.225	c
3.125 +M	3.705	d
1.56 +M	4.155	e
25 -M	12.905	f
12.5 -M	13.055	fg
6.25 -M	13.165	gh
0+M	13.185	gh
3.125 -M	13.295	gh
1.56 -M	13.375	hi
0 -M	13.595	i

Duncan's multiple range test
Pure glucosinolates x Concentration (µmol) × Myrosinase

	Mean	
Gluconapin 25 +M	2.100	a
Gluconasturtiin 25 +M	2.280	ab
Gluconapin 12.5 +M	2.320	abc
Mixed GSL 25 +M	2.440	abc
Glucoraphanin 25 +M	2.520	abc
Gluconasturtiin 12.5 +M	2.680	bcd
Mixed GSL 12.5 +M	2.820	cde
Gluconapin 6.25 +M	3.040	def
Mixed GSL 6.25 +M	3.060	def
Glucoraphanin 12.5 +M	3.060	def
Gluconasturtiin 6.25 +M	3.340	efg
Glucoraphanin 6.25 +M	3.460	fgh
Gluconapin 3.125 +M	3.460	fgh
Mixed GSL 3.125 +M	3.680	ghi
Gluconasturtiin 3.125 +M	3.720	ghi
Gluconapin 1.56 +M	3.860	ghi
Glucoraphanin 3.125 +M	3.960	hi
Gluconasturtiin 1.56 +M	4.060	i
Mixed GSL 1.56 +M	4.080	i
Glucoraphanin 1.56 +M	4.620	j
Glucoraphanin 25 -M	12.660	k
Glucoraphanin 12.5 -M	12.880	kl
Gluconasturtiin 25 -M	12.960	klm
Gluconapin 25 -M	12.960	klm
Glucoraphanin 6.25 -M	12.980	klmn
Mixed GSL 25 -M	13.040	klmn
Glucoraphanin 3.125 -M	13.080	klmno
Gluconasturtiin 12.5 -M	13.080	klmno

Gluconapin 12.5 -M	13.100	klmnop
Glucoraphanin 0+M	13.120	klmnop
Mixed GSL 12.5 -M	13.160	klmnop
Gluconasturtiin 0+M	13.180	klmnop
Mixed GSL 0+M	13.200	klmnop
Gluconasturtiin 6.25 -M	13.200	klmnop
Gluconapin 6.25 -M	13.200	klmnop
Glucoraphanin 1.56 -M	13.220	klmnop
Gluconapin 0+M	13.240	lmnop
Mixed GSL 6.25 -M	13.280	lmnop
Gluconasturtiin 3.125 -M	13.300	lmnop
Gluconapin 3.125 -M	13.360	lmnop
Gluconasturtiin 1.56 -M	13.380	lmnop
Mixed GSL 1.56 -M	13.440	lmnop
Mixed GSL 3.125 -M	13.440	lmnop
Gluconapin 1.56 -M	13.460	lmnop
Glucoraphanin 0 -M	13.500	mnop
Gluconasturtiin 0 -M	13.560	nop
Gluconapin 0 -M	13.640	op
Mixed GSL 0 -M	13.680	p

Sorghum halepense shoot weight (mg)

Duncan's multiple range test
Pure glucosinolates

	Mean	
Gluconasturtiin	44.59	a
Gluconapin	47.10	b
Mixed GSL	47.95	b
Glucoraphanin	53.48	c

Duncan's multiple range test
Concentration (µmol)

Mean		
25	37.96	a
12.5	41.25	b
6.25	43.85	c

3.125	47.52	d
1.56	51.88	e
0	67.22	f

Duncan's multiple range test
Pure glucosinolates x Concentration (µmol)

	Mean	
Gluconasturtiin 25	32.55	a
Gluconapin 25	37.10	b
Gluconasturtiin 12.5	38.10	b
Gluconapin 12.5	39.80	bc
Mixed GSL 25	40.10	bcd
Gluconasturtiin 6.25	40.20	bcd
Glucoraphanin 25	42.10	cde
Mixed GSL 12.5	42.20	cde
Gluconapin 6.25	42.80	cde
Gluconasturtiin 3.125	43.40	def
Mixed GSL 6.25	44.40	efg
Glucoraphanin 12.5	44.90	efgh
Gluconapin 3.125	46.20	fghi
Mixed GSL 3.125	46.80	ghij
Glucoraphanin 6.25	48.00	hijk
Gluconasturtiin 1.56	48.30	ijk
Gluconapin 1.56	49.50	jk
Mixed GSL 1.56	50.70	k
Glucoraphanin 3.125	53.70	l
Glucoraphanin 1.56	59.00	m
Mixed GSL 0	63.50	n
Gluconasturtiin 0	65.00	no
Gluconapin 0	67.20	o
Glucoraphanin 0	73.20	p

Duncan's multiple range test
Pure glucosinolates x Myrosinase

	Mean	
Gluconasturtiin +M	31.88	a
Gluconapin +M	33.90	b
Mixed GSL +M	37.67	c
Glucoraphanin +M	39.47	d
Gluconasturtiin -M	57.30	e
Mixed GSL -M	58.23	e
Gluconapin -M	60.30	f
Glucoraphanin -M	67.50	g

Duncan's multiple range test
Concentration (µmol) × Myrosinase

	Mean	
25 +M	21.78	a
12.5 +M	25.35	b
6.25 +M	28.65	c
3.125 +M	33.55	d
1.56 +M	38.90	e
25 -M	54.15	f
12.5 -M	57.15	g
6.25 -M	59.05	g
3.125 -M	61.50	h
1.56 -M	64.85	i
0+M	66.15	i
0 -M	68.30	j

Duncan's multiple range test
Pure glucosinolates x Concentration (µmol) × Myrosinase

	Mean	
Gluconasturtiin 25 +M	17.90	a
Gluconapin 25 +M	20.40	ab
Gluconasturtiin 12.5 +M	21.60	ab
Glucoraphanin 25 +M	21.80	ab
Gluconapin 12.5 +M	23.60	bc
Gluconasturtiin 6.25 +M	24.60	bcd
Glucoraphanin 12.5 +M	26.40	cde
Mixed GSL 25 +M	27.00	cde
Gluconapin 6.25 +M	27.40	cde
Gluconasturtiin 3.125 +M	28.80	def
Mixed GSL 12.5 +M	29.80	ef
Glucoraphanin 6.25 +M	30.20	efg
Gluconapin 3.125 +M	30.80	efgh
Mixed GSL 6.25 +M	32.40	fgh
Gluconapin 1.56 +M	34.40	gh
Gluconasturtiin 1.56 +M	34.80	h
Mixed GSL 3.125 +M	35.20	h
Glucoraphanin 3.125 +M	39.40	i
Mixed GSL 1.56 +M	39.60	i
Glucoraphanin 1.56 +M	46.80	j
Gluconasturtiin 25 -M	47.20	j
Mixed GSL 25 -M	53.20	k
Gluconapin 25 -M	53.80	kl
Mixed GSL 12.5 -M	54.60	kl
Gluconasturtiin 12.5 -M	54.60	kl
Gluconasturtiin 6.25 -M	55.80	kl
Gluconapin 12.5 -M	56.00	kl
Mixed GSL 6.25 -M	56.40	kl
Gluconasturtiin 3.125 -M	58.00	lm

Gluconapin 6.25 -M	58.20	lm
Mixed GSL 3.125 -M	58.40	lm
Gluconapin 3.125 -M	61.60	mn
Gluconasturtiin 1.56 -M	61.80	mn
Mixed GSL 1.56 -M	61.80	mn
Mixed GSL 0+M	62.00	mno
Glucoraphanin 25 -M	62.40	mno
Glucoraphanin 12.5 -M	63.40	nop
Gluconasturtiin 0+M	63.60	nop
Gluconapin 1.56 -M	64.60	nop
Mixed GSL 0 -M	65.00	nop
Glucoraphanin 6.25 -M	65.80	nop
Gluconasturtiin 0 -M	66.40	nop
Gluconapin 0+M	66.80	opq
Gluconapin 0 -M	67.60	pq
Glucoraphanin 3.125 -M	68.00	pqr
Glucoraphanin 1.56 -M	71.20	qrs
Glucoraphanin 0+M	72.20	rs
Glucoraphanin 0-M	74.20	s

Sorghum halepense Root weight (mg)

Duncan's multiple range test
Pure glucosinolates

	Mean	
Gluconasturtiin	3.687	a
Glucoraphanin	3.710	a
Mixed GSL	3.975	b
Gluconapin	3.977	b

Duncan's multiple range test
Concentration (µmol)

	Mean	
25	3.110	a
12.5	3.312	b
6.25	3.542	c

3.125	3.817	d
1.56	4.280	e
0	4.960	f

Duncan's multiple range test
Pure glucosinolates x Concentration (µmol)

	Mean	
Glucoraphanin 25	2.970	a
Gluconasturtiin 25	3.030	ab
Gluconapin 25	3.200	abc
Gluconasturtiin 12.5	3.230	bcd
Mixed GSL 25	3.240	bcd
Glucoraphanin 12.5	3.270	bcd
Mixed GSL 12.5	3.370	cde
Gluconapin 12.5	3.380	cdef
Glucoraphanin 6.25	3.430	cdefg
Gluconasturtiin 6.25	3.480	defg
Gluconapin 6.25	3.590	efg
Glucoraphanin 3.125	3.640	fg
Mixed GSL 6.25	3.670	g
Gluconasturtiin 3.125	3.690	g
Gluconapin 3.125	3.950	h
Gluconasturtiin 1.56	3.950	h
Mixed GSL 3.125	3.990	h
Glucoraphanin 1.56	4.110	h
Mixed GSL 1.56	4.420	i
Gluconapin 1.56	4.640	ij
Gluconasturtiin 0	4.740	j
Glucoraphanin 0	4.840	j
Gluconapin 0	5.100	k
Mixed GSL 0	5.160	k

Duncan's multiple range test
Pure glucosinolates x Myrosinase

	Mean	
Gluconasturtiin +M	2.653	a
Glucoraphanin +M	2.697	a
Gluconapin +M	2.997	b
Mixed GSL +M	3.037	b
Gluconasturtiin -M	4.720	c
Glucoraphanin -M	4.723	c
Mixed GSL -M	4.913	d
Gluconapin -M	4.957	d

Duncan's multiple range test
Concentration (µmol) × Myrosinase

	Mean	
25 +M	1.675	a
12.5 +M	1.985	b
6.25 +M	2.310	c
3.125 +M	2.755	d
1.56 +M	3.545	e
25 -M	4.545	f
12.5 -M	4.640	fg
6.25 -M	4.775	gh
0+M	4.805	gh
3.125 -M	4.880	hi

1.56 -M	5.015	ij
0 -M	5.115	j

Duncan's multiple range test

Pure glucosinolates x Concentration (µmol) × Myrosinase

	Mean	
Glucoraphanin 25 +M	1.440	a
Gluconasturtiin 25 +M	1.520	ab
Gluconasturtiin 12.5 +M	1.840	bc
Gluconapin 25 +M	1.840	bc
Mixed GSL 25 +M	1.900	cd
Glucoraphanin 12.5 +M	1.960	cd
Mixed GSL 12.5 +M	2.060	cde
Gluconapin 12.5 +M	2.080	cde
Glucoraphanin 6.25 +M	2.200	cdef
Gluconasturtiin 6.25 +M	2.240	defg
Gluconapin 6.25 +M	2.380	efg
Mixed GSL 6.25 +M	2.420	efg
Glucoraphanin 3.125 +M	2.540	fgh
Gluconasturtiin 3.125 +M	2.600	gh
Gluconapin 3.125 +M	2.880	hi
Mixed GSL 3.125 +M	3.000	i
Gluconasturtiin 1.56 +M	3.020	i
Glucoraphanin 1.56 +M	3.380	j
Mixed GSL 1.56 +M	3.780	k
Gluconapin 1.56 +M	4.000	k
Glucoraphanin 25 -M	4.500	l
Gluconasturtiin 25 -M	4.540	lm
Gluconapin 25 -M	4.560	lm
Glucoraphanin 12.5 -M	4.580	lmn
Mixed GSL 25 -M	4.580	lmn
Gluconasturtiin 12.5 -M	4.620	lmno

Glucoraphanin 0+M	4.660	lmnop
Glucoraphanin 6.25 -M	4.660	lmnop
Gluconapin 12.5 -M	4.680	lmnop
Mixed GSL 12.5 -M	4.680	lmnop
Gluconasturtiin 0+M	4.700	lmnop
Gluconasturtiin 6.25 -M	4.720	lmnop
Glucoraphanin 3.125 -M	4.740	lmnop
Gluconasturtiin 0 -M	4.780	lmnop
Gluconasturtiin 3.125 -M	4.780	lmnop
Gluconapin 0+M	4.800	lmnop
Gluconapin 6.25 -M	4.800	lmnop
Glucoraphanin 1.56 -M	4.840	lmnop
Gluconasturtiin 1.56 -M	4.880	lmnopq
Mixed GSL 6.25 -M	4.920	mnopqr
Mixed GSL 3.125 -M	4.980	nopqr
Glucoraphanin 0 -M	5.020	opqrs
Gluconapin 3.125 -M	5.020	opqrst
Mixed GSL 0+M	5.060	pqrstu
Mixed GSL 1.56 -M	5.060	pqrstu
Mixed GSL 0 -M	5.260	qrstu
Gluconapin 1.56 -M	5.280	rstu
Gluconapin 0 -M	5.400	su