Bioherbicidal properties of sunflower (*Helianthus annuus* L.) and its activities in weed management

Thesis submitted for the degree of Doctor of Philosophy

By

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Declaration

I, Saber Wasman Hamad, hereby declare that the content of this thesis is my own and has not been published or accepted in any previous application for any degree or qualification before. All sources used in the thesis have been specifically acknowledged within the content of the document.

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Dedication

This thesis is gratefully dedicated to my beloved parents, my father Wasman and my mother Fatima, and my beloved brothers and sisters

Abstract

The use of chemical herbicides to suppress weeds possess risks to environment, health, water contamination, and soil microorganisms. It is therefore imperative that research into more eco-friendly alternatives is conducted. Furthermore, there are more than 470 biotypes of weeds that are resistant to chemical herbicides. Sunflower (*Helianthus annuus*) is one of the most important oil crops globally. It produces strong allelochemical compounds that have been shown to affect some crops and weeds. This study was conducted in order to investigate bioherbicidal properties of sunflower on germination and growth of some crop and weed species. The study consisted of five experiments.

The first experiment was conducted using petri dishes in order to evaluate effects of sunflower growth stage on the bioherbicidal activity of aqueous sunflower shoot and root extracts on seed germination and early growth of *Brassica napus*, *Secale cereale*, *Cephalaria syriaca*, *Phalaris minor*, *Pisum sativum*, *Triticum aestivum*, *Avena fatua* and *Helianthus annuus***.** Extracts were prepared from dried shoots and roots of sunflower sampled at one week of growth, and at one and two months of age and also at crop maturity. Aqueous two month shoot extracts caused a significantly higher reduction (81.27%) in seed germination and seedling growth than one week (54.44%), one month (55.67%) and mature stage extracts (62.75%) of most seed species. On the other hand, one month root extract had a more negative impact on seed germination (54.17%) and seedling growth than other root extracts.

Also the dry matter yield of sunflower shoot and root extracts at different growth stages was studied. Two month shoot (18.9%) and one week root extracts (14.22%) gave higher yields than other extracts of each type. Analysis of phytochemicals from sunflower shoot and root extracts at different growth stages indicated that tannins, terpenoids, saponins, and phenolics were present in both shoot and root extracts at most growth stages while phlobataninns were only present in root extracts at different growth stages except one week.

A second experiment was carried out in order to investigate the allelopathic potential of different concentrations of aqueous shoot extracts prepared from shoots of plants at the mature stage on seed germination and seedling growth of two monocot (*S. cereale*, *P. minor*) and two dicot (*B. napus, C. syriaca*) plant species. Seed germination and seedling growth of *B. napus* and *C. syriaca* were completely inhibited at 5% and 10% (w/v) concentrations respectively.

The effect of sunflower shoot extract on mitotic index and cell elongation was investigated. Aqueous sunflower shoot extract significantly reduced root cell elongation (control: 8.2 µm, test: 6.2 µm) of *A. fatua.* However, statistical analysis indicated that aqueous sunflower shoot extract did not significantly affect mitotic index. No significant difference in distribution of cells between mitotic phases (prophase, metaphase, anaphase, and telophase) was recorded.

The third experiment involved pot experiments for examination of the allelopathic effects of two month sunflower shoot aqueous extract and incorporated two month sunflower ground shoot on seed germination, shoot and root length, shoot and root dry weight, chlorophyll a and chlorophyll b of *B. napus, A. fatua, C. syriaca* and *P. minor* grown in soil. Soil calcium, magnesium, potassium, pH and electrical conductivity were also measured. In general, two month sunflower ground shoot caused more significant reduction in seed germination and early growth than shoot aqueous extract.

The fourth experiment involved identification and quantification of phenolic compounds present in sunflower shoot and root extracts. Four phenolic compounds, syringic acid, protocatechuic acid, 4-hydroxybenzoic acid, and ferulic acid, present in root extracts from one week old sunflowers were identified and quantified. Additionally, twelve phenolic compounds were identified and quantified in sunflower shoot extracts from one month, two month and mature stage plants. These were gallic acid, syringic acid, vanillic acid, protocatechuic acid, catechol, 4-hydroxybenzoic acid, p-coumaric acid, sinapic acid, ferulic acid, caffeic acid, chlorogenic acid, and trans-cinnamic acid. Sunflower two months shoot extract had the greatest concentration of phenolic compounds (0.026 mg/ml) compared with those from other growth stages.

The effects of total and individual phenolic compounds on seed germination and seedling growth of *B. napus, C. syriaca, T. aestivum* and *S. cereale* were investigated. Total phenolic compounds caused the greatest reduction in seed germination and seedling growth followed by chlorogenic acid and caffeic acid.

For the fifth experiment, the effects of a positive control, the herbicide trifluralin, on seed germination and growth of *B. napus* and *C. syriaca* were examined at different concentrations in petri dishes. Seed germination of both species was significantly decreased by most concentrations ($P < 0.001$). The inhibition percentage of seed germination ranged between 21 and 32%. The highest reduction of seed germination (32.28%) was from the highest trifluralin concentration (900 ppm).

The effects of an aqueous shoot extract from two month old sunflower plants, the total phenolic compounds identified within these extracts and trifluralin on sugar content, protein content, proline content, DNA content, gibberellic acid (GA) content, indole acetic acid (IAA) content, and abscisic acid (ABA) content of *B. napus*, *C. syriaca*, *T. aestivum* and *S. cereale* seedlings were examined. Trifluralin reduced sugar content significantly more than sunflower extract did ($P < 0.001$). Both trifluralin and sunflower extract significantly increased protein content compared to the control treatment ($P < 0.001$). Total phenolic compounds significantly reduced proline content in *S. cereale* and *C. syriaca*; the effect of trifluralin was only significant for *C. syriaca* ($P < 0.001$). Trifluralin had the greatest effect on DNA content of *T. aestivum* and *S. cereale* and total phenolic compounds the least in comparison to the control treatment ($P < 0.001$). Sunflower extract had the greatest effect on GA contents. ABA was reduced by application of total phenolic compounds and sunflower shoot extracts in *S. cereale* and *C. syriaca.* Sunflower shoot aqueous extract and total phenolic compounds significantly reduced IAA in all studied plant species but trifluralin only reduced IAA in *T. aestivum*, *B. napus* and *C. syriaca* ($P < 0.001$).

A pot experiment was carried out for examination of the effects of sunflower ground shoot, its total phenolic compounds, and trifluralin on seed germination and seedling growth of *S. cereale, T. aestivum, B. napus* and *C. syriaca*. Trifluralin had a significantly greater effect on seed germination of most plant species. The inhibition percentage ranged between 26 and 100% for shoot and root length and shoot and root dry weight, while total phenolic compounds had the least effect ($P < 0.001$).

In conclusion, sunflower shoot extract from two month old plants has a significantly greater influence on seed germination and seedling growth than other extracts. Also, the application of total phenolic compounds causes significantly more reduction in seed germination and early growth. Two month sunflower ground shoot has a greater effect on most parameters measured than aqueous sunflower shoot extract in pot experiments. Furthermore, the effects of sunflower two month ground shoot, total phenolic compounds and trifluralin in pot experiments indicated that trifluralin has the greatest effect on seed germination and seedling growth while total phenolic compounds have least effect on seed germination.

List of abbreviations

Abbreviation	Description
ABA	Abscisic acid
ANOVA	Analysis of variance
DNA	Deoxyribonucleic acid
dS/m	decisiemens/m
EC	Electrical conductivity
FW	Fresh weight
g	Gravity
GA	Gibberellic acid
HPLC	High performance Liquid Chromatography
h	Hour
i.e.	That is
IAA	Indole acetic acid
min	Minute
N	Normality
rpm	Revolutions per minute
TFA	Trifluoroacetic acid
w:v	Weight: volume

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CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

1.1 Introduction

The phenomenon of plants affecting other neighbouring plants through releasing chemicals was originally mentioned as early as 370 BC by Theophrastus (Zeng *et al.*, 2008). The term Allelopathy was first mentioned by Molisch (1937). It is a Greek hybrid word, "Allelon", which means "of each other" and "pathos" meaning "suffer" (Rizvi *et al.*, 1992). Allelopathy can be defined as any direct or indirect harmful or beneficial effect of one plant or a microorganism on other plants by releasing chemicals termed allelochemicals to the environment (Rice, 1984; Dayan and Duke, 2009). According to the International Allelopathy Society (International Allelopathy Society, 1996), allelopathy is "any process involving secondary metabolites produced by plants, algae, bacteria and fungi that influences the growth and development of agricultural and biological systems".

Many secondary plant products are linked with allelopathic influences. Some secondary plant metabolites, such as phenolics and alkaloids, play an essential role in natural plant activities such as germination and early growth (Inderjit, 1996; Bogatek *et al.*, 2006; Belz, 2007; Dayan and Duke, 2009). Plants which have allelopathic potential must produce allelochemicals, which must be released into the environment and must be available for transport to the target plant to be taken up (Olofsdotter *et al.*, 2002). Allelochemicals are released to neighbouring plants by different mechanisms involving root exudation, leaching, volatilization and decomposition of plant residue (Weston and Duke, 2003).

Allelopathy in plant interactions may have many complicated relations with the environment involving competition, stimulation, inhibition, and interdependence. Some field grown plants grow in a mixture with many plant species, but others grow in monocultures. The influence of one plant on another neighbouring plant is called interference, which involves allelopathy and competition. Competition is the ability of a plant to gain advantage over other plants by obtaining limited resources from the environment more effectively, whereas allelopathy refers to plants that have allelochemicals that affect other plant species in the same environment (Rice, 1974; Zeng *et al.*, 2008).

1.2 Field Crop Production

Certain crop species can be used as allelopathic plants and their allelochemical extracts can be used to advantage to suppress and reduce negative effects of weeds on crop production (Belz, 2007). Such an approach could also help to avoid environmental pollution and to reduce concerns about effects on human health and soil contamination associated with the use of synthetic herbicides (Subtain *et al.*, 2014). Crop allelopathy, i.e. the ability of a crop species to exert allopathic effects, could be achieved by using such crops as cover crops, companion crops, or smother crops as well as components of the crop rotation system. Selecting crops to release allelochemicals may minimize the intensity not only of weeds, but also pests, diseases and nematodes (Khanh *et al.*, 2005).

Crop allelopathy may play an essential role in the development of biological herbicides: for example, Macías *et al.* (2004) developed bioherbicides containing phenolics and terpenes as active constituents from sunflower. It may also contribute to a more sustainable agriculture by reducing environmental deterioration associated with synthetic agrochemicals as mentioned previously and improving soil quality when crop residues decompose (Xuan *et al.*, 2005).

Some weeds have been shown to have allelopathic properties against some crop plants. For example, *Asphodelus tenuifolius* and *Fumaria indica* have been shown to have negative impacts on germination and germination index of *Zea mays* (Jabeen and Ahmed, 2009).

1.3 Sunflower and allelopathy

Sunflower (*Helianthus annuus* L.) is one of the most important oil crops globally (Kaya *et al.*, 2006). It produces strong allelochemical compounds that have been shown to affect some crops and weeds (Leather, 1983). Some allelopathic compounds have been isolated from sunflower plants and identified, including phenolics, triterpenes, steroids, flavonoids, heliespirones, heliannuoles, sesquiterpenes and helikauranoside A (Macías *et al.*, 2002a). Recently, studies on allelochemicals in sunflower showed the crop's potential importance for sustainable weed control (Anjum *et al.*, 2005). Sunflower aqueous extract has inhibitory effects on shoot and root growth of some plants and weeds (Ghafar *et al.*, 2000).

Macías *et al.* (2002b) isolated about 125 natural allelochemical compounds from different sunflower extracts and investigated their effects on germination and growth of numerous weed species. Moreover, there have been many investigations of the effect of sunflower

allelochemicals on germination and growth of many monocotyledon and dicotyledonous plants (Macías *et al.*, 2000).

Ghafar *et al.* (2001) identified five phenolic compounds (chlorogenic, caffeic, syringic, vanillic and ferulic acids) in sunflower aqueous leaf extract, three phenolic acids (chlorogenic, ferulic and vanillic acids) from sunflower aqueous stem extract and one from sunflower aqueous root extract (ferulic acid). The study also indicated that sunflower aqueous extracts from leaves contained more total phenolic compounds than stems and roots.

Alsaadawi *et al.* (2012) investigated effects of eight sunflower cultivars on germination and growth of wheat and their accompanying weeds. All sunflower residues incorporated into soil significantly reduced total number and biomass of studied weeds. Extracts of sunflower Sin-Altheeb and Coupon genotypes had greater suppressive effects on weeds than other cultivars. Furthermore, 13 allelochemical compounds, most of which were phenolic acids (chlorogenic acid, isochlorogenic acid, caffeic acid, gallic acid, protocatecheic acid, syringic acid, hydroxybenzoic acid, p-coumaric acid, ferulic acid, vanillic acid, catechol, sinapic acid and terpinol) were isolated from sunflower extracts by using HPLC.

Kamal (2010) examined allelopathic effects of sunflower leaf, stem and root (1 g/ 10 ml distilled water) aqueous extracts on growth and physiological processes of two varieties of wheat (Margalla 99 and Chakwa1l 97) and some weeds in pot experiments. Sunflower leaf aqueous extract significantly decreased weed fresh and dry weight compared with the untreated control. GA and IAA were also affected by sunflower leaf, stem and root aqueous extracts. The highest concentration of GA was recorded with the control treatment followed by stem extract treatment, while the minimum concentration was recorded with leaf extract treatment. However, ABA content was increased by the application of sunflower leaf extract followed by root extract while the control showed the minimum concentration of ABA in seedlings of both wheat varieties.

Bradosti (2007) studied the effect of sunflower shoot straw at different concentrations (1120, 2240, and 4440 kg/ha), incorporated into soil in a field experiment, on seed germination and growth of sunflower, maize, soybean and three accompanying weeds (*Echinochloa colonum*, *Amaranthus retroflexus*, and *Portulaca oleracea*). Sunflower shoot straw at the highest concentration significantly reduced seed germination percentage, sunflower disc diameter, the weight of hundred seeds, and yield of sunflower plants. Sunflower shoot straw reduced seed germination percentage, stem diameter, leaf area index, plant height, the weight of hundred

grains of maize, while for soybean, sunflower shoot straw significantly reduced seed germination percentage, number of branches/plant, the number of pods/plant, the number of seeds/pod, and the weight of hundred seeds. Also sunflower shoot straw significantly reduced growth of *Echinochloa colona* and *Amaranthus retroflexus*.

1.4 Weed Management

Weeds are defined as plants growing in unwanted locations which compete with other plants for resources such as water, nutrients, and light, reduce the yield and quality of crops and may contaminate produce with weed seeds. About 7000 weed species have been identified. Nearly 200-300 of them are problems for farmers (Macias *et al.*, 1995) so weed control is essential in agriculture systems. However, using herbicides to minimize the negative impact of weeds on crop yield has many risks.

Recently, results of some research have shown that using chemical herbicides to suppress weeds, poses risks to environment, health, water contamination, and soil microorganisms (Nikneshan *et al.*, 2011a). Furthermore, there are more than 470 biotypes of weeds that are resistant to chemical herbicides (Heap, 2017). Alternatively, to avoid these potential problems, plants that have allelopathic activity can be used as bioherbicides for weed suppression, so that allelopathy may be considered as a possible tool to minimize weeds and enhance crop production (Cheema and Khaliq, 2000; Thahir and Ghafoor, 2011). According to Batish *et al.* (2001), 35 crop species have been identified to have negative impacts on weed growth.

Minimizing growth of weeds by neighbouring crop plants is a combination of allelopathy and physical interactions, which includes competition for light, water, nutrients and other interactions from the cover crop residue or living mulch. Usually, allelopathic research is conducted in greenhouses and does not account for the impact of climate, microorganisms, and type of soil, and may overstate the potential weed control compared to field conditions. In field conditions, the impact of physical interference is difficult to recognise and to separate from allelopathic effects. Nevertheless, some studies have demonstrated allelopathic impacts of cover crops on weed plants in the field (Colquhoun, 2006). The magnitude of the effect of decreasing weed growth depends on the cover crop cultivar, the amount and thickness of the incorporated layer and management type (Creamer *et al.*, 1996).

1.5 Soil and allelopathy

According to Alan (1993), soil is a system which gives a living biological environment for living microorganisms such as fungi, bacteria, algae, protozoa and actinomycetes. Soil is the environment where allelopathic activities happen. Soil type significantly affects the allelopathic potential of allelochemicals (Teasdale *et al.*, 2012). Allelopathic activity of several allelochemicals can be reduced by organic matter, ion exchange capacity, inorganic ions, and mineral reactive surfaces as well as abiotic and biotic factors during application in soil (Blum *et al.*, 1993; Schmidt *et al.*, 2000; Inderjit, 2001; Hiradate *et al.*, 2010).

Allelochemicals incorporated into soil may be transformed when movement happens and they are metabolized by soil microbes (Cheng, 1995; Inderjit, 2001). Biotic and abiotic factors in soil affect allelochemicals: for example, phenolic impounds may be transformed to non-toxic phenolics (Cheng, 1995; Huang *et al.*, 1999). Chou and Leu (1992) showed that for an aqueous extract from *Delonix regia*, rhizosphere phenolic acids were water soluble and were leached to depth in the soil. Another study investigated the relation between phenolic acids and soil nutrients and indicated that phenolic acids influence nutrient availability in soil (Rice, 1984). However, few studies have examined the effect of soil on allelochemicals (Dakshini and Dakshini, 1996; Inderjit and Nishimura, 1999). According to Kobayashi (2004) allelochemicals are complex and susceptible to the effects of soil conditions. Furthermore, soil adsorbs allelochemicals and metabolizes them via chemical and biological reactions. Also, some factors affect their behaviour, including soil texture, organic and inorganic matter, soil moisture content and microorganisms. Phytochemical activity of the substances produced and released from plant straws has been shown to be influenced by environmental aspects - for example, soil physiochemical properties, soil microbes, and nutrient concentrations in soil (Kitou and Okuno, 1999; Sène *et al.*, 2000).

Soil pH affects availability of soil nutrients and hence plant growth (Chou, 1989; Vessey *et al.*, 1990; Chaillou *et al.*, 1991). Allelochemicals may affect soil pH (Souto *et al.*, 2001) and therefore affect plant growth. It has been recognized that phenolic compounds may reduce soil pH due to soil acidification (Sasikumar *et al.*, 2002; Zhang and Fu, 2009). A study by Inderjit and Dakshini (1994) showed that ground shoot of *Pluchea lanceolata* incorporated with soil (sandy-loam) significantly reduced pH but electrical conductivity was increased. Eventually, the shoot straw of *P. lanceolata* significantly affected seed germination and seedling growth of rapeseed (*Brassica napus*).

Electrical conductivity reflects the concentration of materials which are relevant to salinity. High temperature, low relative humidity and long-term salinization in soil can increase damage to plants by salinity (Marchese *et al.*, 2008). Guo *et al.* (2013) studied the effects of ferulic acid and coumarin on germination and growth of *Microcystis aeruginosa.* Ferulic acid and coumarin at higher concentrations (100 mg/L) affected the growth of *M. aeruginosa* but they promoted physiological activity at lower concentrations. Ferulic acid and coumarin at high concentration (200 mg/L) significantly affected electrical conductivity.

Kamal and Bano (2008) investigated the effect of growing sunflower (Hysun 38) plants on soil physiochemical elements (electrical conductivity, pH, Mn, Ca, K, P, and soil moisture). Electrical conductivity was significantly decreased from 130 dS/m to 110dS/m. Soil calcium availability was also decreased from 210.91 to 120.02 ppm. However, Mn, Fe, Mg, K, Zn and pH were significantly increased in soil in which sunflowers had been grown.

1.6 Allelochemicals

The allelopathic compounds present in some plants are mostly secondary metabolites, including phenolics, terpenoids, and alkaloids (Einhellig and Leather, 1988; Kruse *et al.*, 2000). Phenolics and terpenoids are the most common. The concentrations of allelochemicals in plant residues and aqueous extracts are affected by the age of plants, plant stress and environmental conditions (Pedrol *et al.*, 2006). Higher concentrations of these allelochemicals have an inhibitory effect but in contrast, lower concentrations can promote seed germination and seedling growth of plants (Einhellig *et al.*, 1993; Narwal, 1994).

Terpenoids may influence plant seeds and soil microorganisms by leaching, volatilization, or decomposition of plant residues. During the early stages of plant growth, or during stress periods, root exudation by diffusion, ion channels, or residue transport releases many organic and inorganic compounds into the rhizosphere (Battey and Blackbourn, 1993; Uren, 2007). These compounds may help improve nutrient uptake, plant growth regulation, root lubrication, defence against microorganisms and waste removal (Bertin *et al.*, 2003).

There are many crops that produce allelochemicals during their growth, such as sorghum, wheat, alfalfa, barley, corn, asparagus, coffee, tea, tobacco, and sunflower, which can be used as bioherbicides to suppress weeds. Furthermore, these allelopathic crops can produce allelochemicals during decomposition of their plant residues, such as roots and leaves. For example, heliannuols, terpenoids and flavonoids may be released by sunflower plants after decomposition of the plant residue (Macías *et al.*, 1996).

1.6.1 Phenolic compounds as allelochemicals

Phenolic compounds are one of the major groups of plant metabolites which have numerous important functions in plants (Pandey and Rizvi, 2009). They are the most important class of common secondary metabolites which are found in plants and act as allelochemicals in natural ecosystems (Shahidi and Naczk, 1995; Zeng *et al.*, 2008). They originate from the shikimic acid and acetic acid metabolic pathways in plants (Li *et al.*, 2010).

An important class of phenolic compound is the phenolic acids. The primary structures of phenolic acids are benzoic acids and derivatives of cinnamic acids which can be found in different part of plants such as seeds, leaves and roots (Mendoza *et al.*, 2011). Chlorogenic acid is the most well-known phenolic acid, synthesized from caffeic and quinic acids. Phenolic acids have attracted considerable interest because of their numerous health benefits (Breinholt, 1999). They are involved in many industrial functions to produce chemicals, including manufacturing pesticides, explosives, drug production, bleaching process, paper production and dyes. In addition to these functions, the allelopathic activity of phenolic acids (Chou and Lin, 1976; Waller, 1987) may be exploited as non-synthetic pesticides to eliminate weeds, insects and fungi (Mahugo Santana *et al.*, 2009).

Most of the phenolic compounds which have already been identified as allelochemicals are extracted from plant parts, such as shoots and roots. Many years ago, de Candolle (1830) noticed suppressive effects of root exudates on the neighbouring plants (Willis, 1985). However, it was difficult to determine if phenolic compounds were involved in this effect and if so, which ones. After the 1930s there was a revolution: numerous methods such as column chromatography on silica and ion exchange chromatography were developed, which enabled phenolics to be found and extracted from plants (Hostettmann et al., 1997).

Colpas *et al.* (2003) reported that coumaric acid and ferulic acid at concentration 50 mg L^{-1} significantly reduced seed germination and seedling growth of soya bean (*Glycine max*). Another study showed the effect of six phenolic acids (ferulic acid, m-coumaric acid, p-coumaric acid, vanillic acid, p-hydroxybenzoic acid and cinnamic acid) on seed germination and seedling growth of barnyardgrass (*Echinochloa crus-galli*). Cinnamic acid had the greatest effect on shoot (25.41 %) and root length (67.93%), fresh weight (33.32%) and chlorophyll content (73.12%) of barnyardgrass while m-coumaric acid significantly reduced dry weight and seed germination percentage (Esmaeili *et al.*, 2012).

Reigosa *et al.* (1999) carried out a laboratory bioassay investigation of the effect of six phenolic compounds (ferulic acid, gallic acid, p-coumaric acid, p-hydroxybenzoic acid, vanillic acid, and p-vanillin) at different concentrations (10, 1, 0.1, and 0.01 mM) on seed germination and seedling growth of six weed species (*Chenopodium album*, *Plantago lanceolata*, *Amaranthus retroflexus*, *Solanum nigrum*, *Cirsium* sp. and *Rumex crispus*). Results indicated that in general, these phenolic compounds significantly reduced seed germination and seedling growth.

Shaukat *et al.* (1999) investigated the effect of the plant growth regulator 2,4-D and phenolic compounds (benzoic acid, p-coumaric acid, catechol, chlorogenic acid and gallic acid) on seed germination and seedling growth of *Pennisetum americanum* following leaching of these chemicals into the soil in which the seedlings were grown. Benzoic acid gave the most significant reduction in seed germination and shoot and root growth while gallic acid had the least effect. On the other hand, 2,4-D in combination with p-coumaric acid and catechol inhibited germination and seedling growth.

Hussain *et al.* (2010) examined the effects of two phenolic acids (ferulic acid and p-hydroxybenzoic acid) at several concentrations (1.5, 1.0, 0.5, 0.1 mM) and two herbicides (pendimethalin and S-metolachlor) at several concentrations (10^{-1}, 10^{-3}, 10^{-5} and 10^{-7} mM) on growth, physiological activities and carbon isotope discrimination in lettuce (*Lactuca sativa*). Ferulic acid significantly reduced shoot length, leaf length, root length and fresh weight, and quantum efficiency of open photosystem II reaction centres of lettuce. Ferulic acid and p-hydroxybenzoic acid significantly reduced the quantum yield of photosystem II, whereas neither herbicide caused a significant reduction in photosystem II activity.

Ishikura *et al.* (2001) studied the effects of thirteen phenolic compounds (salicylic acid, p-hydroxybenzaldehyde, p-hydroxybenzoic acid, vanillic acid, gallic acid, 3,4-dimethoxybenzoic acid, 3,4,5-trimethoxybenzoic acid, p-coumaric acid, caffeic acid, ferulic acid, ellagic acid, chlorogenic acid and kaempferol) at concentrations of 10^{-5} M, 10^{-4} M, $5x10^{-4}$ M on seed germination and seedling growth of shirakamba birch (*Betula platyphylla* var. japonica). Nine phenolic acids dramatically reduced seed germination percentage and shoot and root growth (60-100% inhibition) of shirakamba birch.

1.6.1.1 Chlorogenic acids

Chlorogenic acids (Figure 1) are produced by combination of cinnamic acids and quinic acid and usually appear in high concentration in comparison with other phenolic acids in many fruits, vegetables, and field crop plants. Chlorogenic acids play an essential role as dietary antioxidants and they are the main polyphenolic acids. Chlorogenic acids include the caffeic acid, ferulic acid and p-coumaric acid mono-esters group, which is the most common group, and various more complex esters (Clifford, 2000). Abdul-Wahab and Al Naib (1972) found that aqueous leaf and stem extracts of *Imperata cylindrica* produced and released chlorogenic acid and isochlorogenic acid (caffeic acid diesters of quinic acid) into the environment.

A study by Chou and Leu (1992) showed that aqueous extracts of flowers, leaves, and twigs of *Delonix regia* reduced the seedling growth of lettuce (*Lactuca sativa*) and Chinese cabbage (*Brassica chinensis*). The phenolic acids chlorogenic acid, protocatechuic acid (3,4-dihydroxybenzoic acid), gallic acid, 3,4-dihydroxybenzaldehyde, p-hydroxybenzoic acid, caffeic acid (3,4-dihydroxycinnamic acid) and 3,5-dinitrobenzoic acid were isolated from *Delonix regia.*

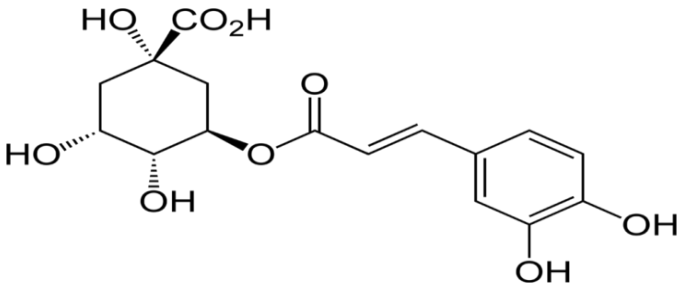


Figure 1 Chemical structure of the chlorogenic acid 5-caffeoylquinic acid

1.6.1.2 Caffeic acid

Caffeic acid (Figure 2) is a well-known important phenolic substance found in plants and is one of the pivotal intermediates of plants, which belongs to hydroxycinnamic acid derivatives (Lin and Yan, 2012). By two steps of sequential hydroxylation at the three and four positions of the benzyl ring, via p-coumaric acid, cinnamic acid is converted into caffeic acid (Kojima and Takeuchi, 1989; Bourgaud *et al.*, 2006). Caffeic acid plays an essential role in inhibiting seed germination and seedling growth of some plants (Chou and Leu, 1992; Barkosky *et al.*, 2000).

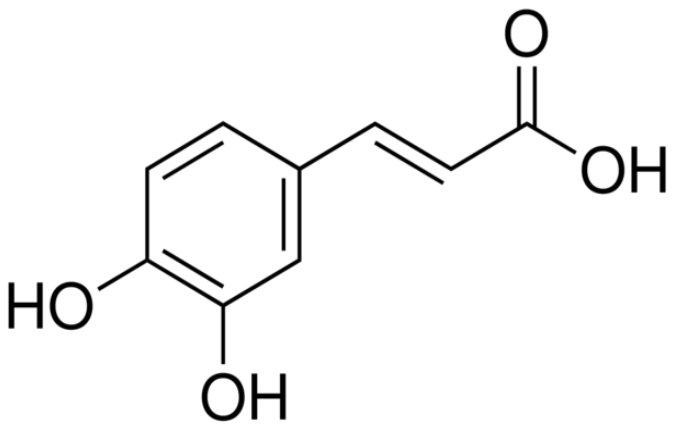


Figure 2 Chemical structure of caffeic acid

1.6.1.3 Trans-cinnamic acid

Cinnamic acid (Figure 3) is a widespread phenolic acid released into the environment by root exudates, leaf leachates and decomposition of plant residues (Yu and Matsui, 1997). Trans-cinnamic acid has a long history of use by humans as a plant component of drugs (Hoskins, 1984). Vaughan and Ord (1991) reported that cinnamic acid affected root morphology of *Pisum sativum.* Another study showed that trans-cinnamic acid damaged cell plasma membranes by affecting sulphydryl groups of soybean (*Glycine max*) (Baziramakenga *et al.*, 1995). Fujita and Kubo (2003) found that trans-cinnamic acid significantly reduced root growth of *Lactuca sativa.*

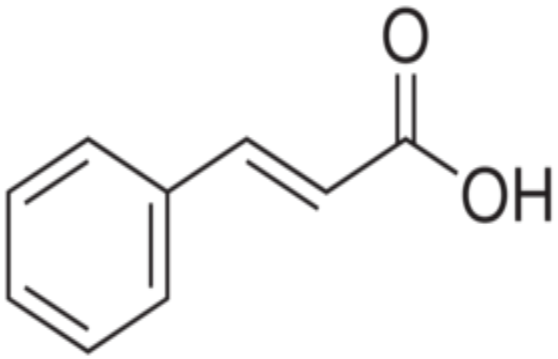


Figure 3 Chemical structure of trans-cinnamic acid

1.6.1.4 p-Coumaric acid

p-Coumaric acid (Figure 4) is a phenolic acid which is a hydroxylated derivative of cinnamic acid (Kojima and Takeuchi, 1989). It is one of the main phenolic acids which is counted as a plant inhibitor and appears in rhizome and leaf extracts (Rice, 1974). p-Coumaric acid plays an essential role in inhibition of seed germination and seedling growth of wheat (Jalageri *et al.*, 2010).

Matok *et al.* (2009) examined the effects of applying walnut leaf extract and different concentrations (0.01 mM, 0.1 mM and 1 mM) of phenolics including p-coumaric acid, chlorogenic acid, catechin, syringic acid, ferulic acid, tannic acid and caffeic acid extracted from walnut on seed germination and seedling growth of dandelion in a soil experiment. Walnut leaf extract inhibited seed germination up to 55%. The highest concentrations (1 mM) completely inhibited seed germination of dandelion while the lowest concentration (0.01 mM) promoted seed germination.

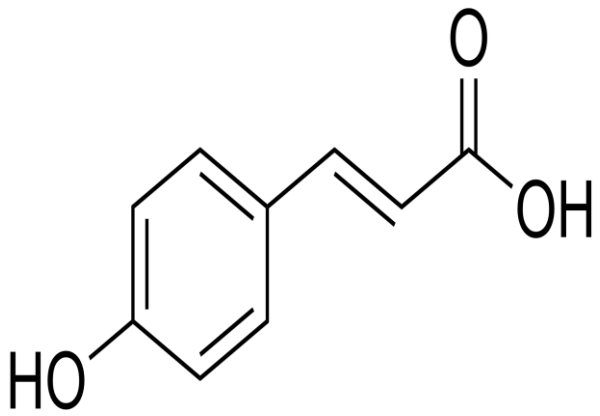


Figure 4 Chemical structure of p-coumaric acid

1.6.1.5 Ferulic acid

Ferulic acid (Figure 5) is also a well-known hydroxyl cinnamic acid derivative which is widely distributed in the plant kingdom. Ferulic acid causes stress in plant roots and influences several physiological and biochemical effects, including utilization of water, foliar expansion, root enlargement, photosynthesis, ion uptake, and respiration (dos Santos and Ferrarese, 2008). A study by Blum and Dalton (1985) revealed that ferulic acid affected leaf area, leaf expansion and oven dry weight of cucumber plants. Another study by Guo *et al.* (2013) showed that ferulic acid at high concentration (100 mg/L) had negative effects on chlorophyll a content of *Microcystis aeruginosa* whereas electrical conductivity was increased. However, lower ferulic concentrations promoted physiological metabolism.

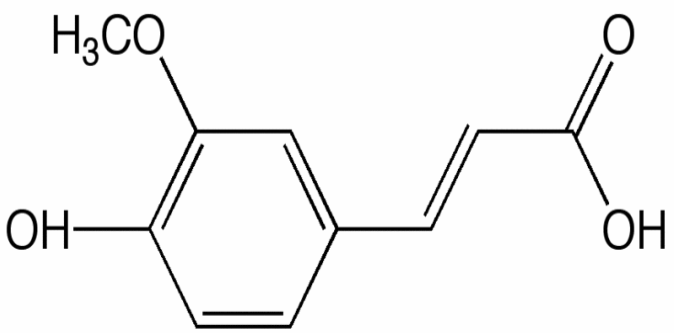


Figure 5 Chemical structure of ferulic acid

1.6.1.6 p-Hydroxybenzoic acid

p-Hydroxybenzoic acid (Figure 6) is a phenolic derivative of benzoic acid released by some plants and involved in allelopathic potential (Rice, 1984). Barkosky and Einhellig (2003) studied the bioherbicidal effect of p-hydroxybenzoic acid on growth and plant water-balance of soybean in greenhouse conditions. p-Hydroxybenzoic acid significantly reduced soybean growth at a concentration of 0.5 mM and at a higher concentration (0.75 mM) reduced stomatal conductance and water potential.

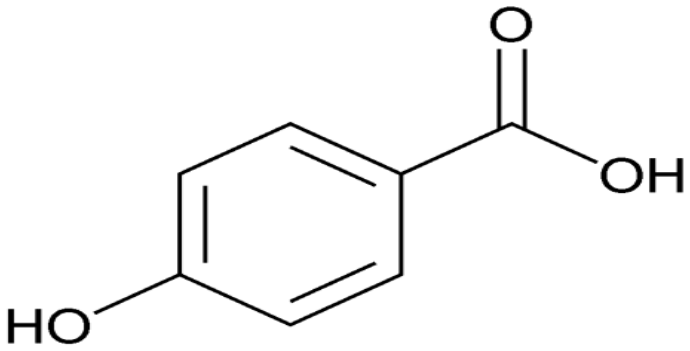


Figure 6 Chemical structure of p-hydroxybenzoic acid

1.6.1.7 Gallic acid

Gallic acid (Figure 7) is a natural phenolic acid (trihydroxybenzoic acid) which is also called 3,4,5-trihydroxybenzoic acid. Xu and Long (2009) separated and identified the compound from black mangrove (*Aegiceras corniculatum*) and also reported it has an allelopathic effect against plants. Similarly, it was reported that gallic acid is one of the substances found in *Polygonella myriophylla* that has allelopathic potential against plants (Weidenhamer and Romeo, 2005). Yang *et al.* (2005) indicated that gallic acid, vanillin, catechin, and other phenolic acids have a dramatic negative effect on growth of the dinoflagellate *Alexandrium tamarense.*

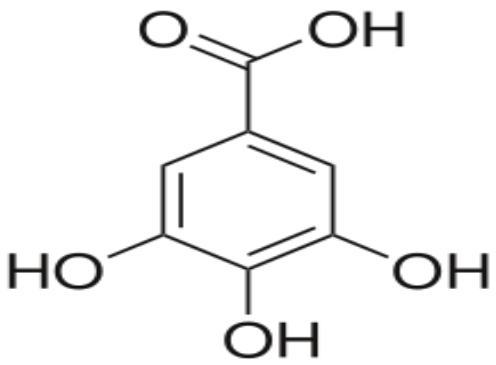


Figure 7 Chemical structure of gallic acid

1.6.1.8 Syringic acid

Syringic acid (Figure 8) is a natural phenolic acid produced by plants as a secondary metabolite. It has been reported that syringic acid in barley (*Hordeum vulgare*) has auto toxicity (Oueslati *et al.*, 2009). Abbas *et al.* (2014) mentioned that weeds of rice produce syringic acid, which can act as a bioherbicidal compound and affect growth of wheat.

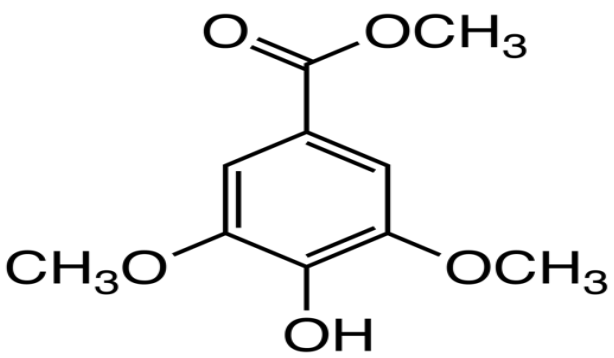


Figure 8 Chemical structure of syringic acid

1.6.1.9 Vanillic acid

Vanillic acid (Figure 9) is a natural hydroxycinnamic acid derivative in plants (Khoddami *et al.*, 2013). It was noticed that vanillic acid may act as a major allelopathic component that has plant-selective activity as examined on seed germination of watermelon (Kushima *et al.*, 1998). Abdaoui (1991) examined the effects of vanillic, ferulic, and gallic acid on seed germination and seedling growth of maize (*Zea mays*), radish (*Raphanus sativus*) and peanut (*Arachis hypogaea*). Results showed that seed germination was significantly inhibited by application of vanillic and ferulic acid and also root elongation and dry weight were affected by higher concentrations of these phenolic acids.

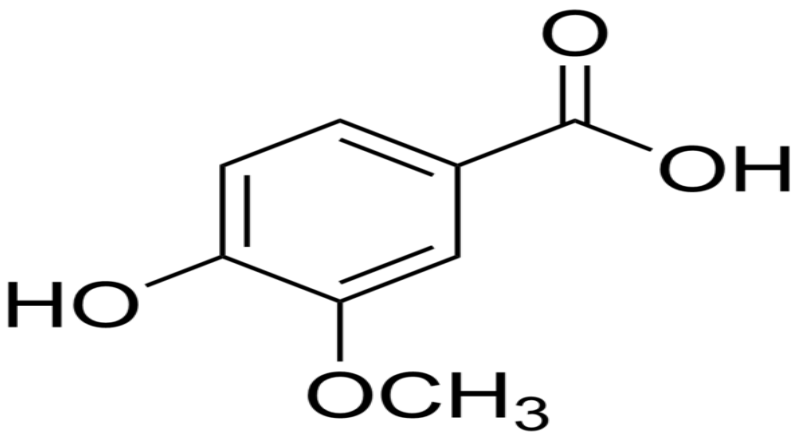


Figure 9 Chemical structure of vanillic acid

1.6.1.10 Protocatechuic acid

Protocatechuic acid (Figure 10) is a natural phenolic acid compound. It has antioxidant and anti-inflammatory properties (Liu *et al.*, 2002) and has been found to have allelopathic potential against germination and growth of plants (Rice, 1984; Siqueira *et al.*, 1991; Blum *et al.*, 1999). Studies showed that protocatechuic acid has an inhibitory effect on hydraulic conductivity, ion uptake, photosynthesis, and transpiration but increases ABA (Blum and Dalton, 1985; Blum and Rebbeck, 1989; Blum, 1995).

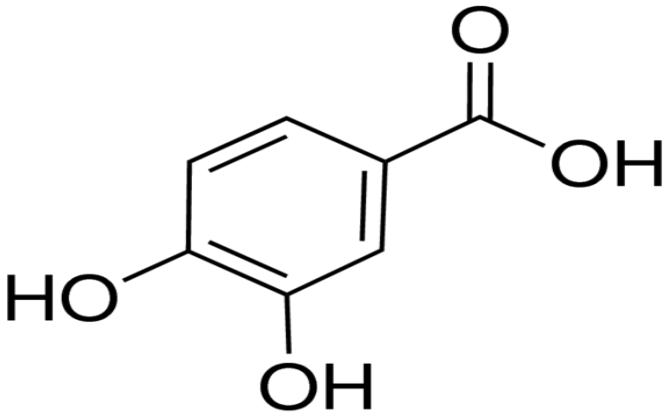


Figure 10 Chemical structure of protocatechuic acid

1.6.1.11 Catechol

Catechol (Figure 11) is a phenolic compound which acts as an allelochemical and is synthesized in plants from shikimic acid via chlorogenic acid. A study conducted by Topal *et al.* (2006), showed the effect of catechol on growth of some weed species (*Papaver rhoeas*, *Cirsium arvense*, *Lamium amplexicaule* and *Sinapis arvensis*) using *Triticum vulgare* and *Hordeum vulgare* as controls. Catechol at 13.64 mM significantly inhibited growth of the studied weeds and the most affected weed was *Papaver rhoeas* in comparison with the control and treatment with the herbicide 2,4-D.

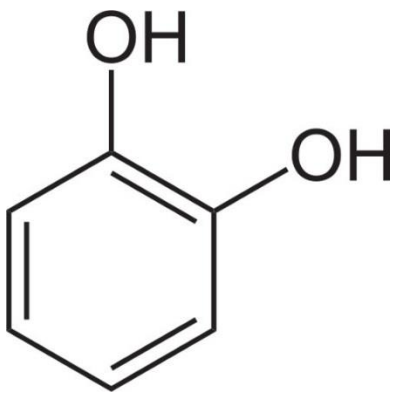


Figure 11 Chemical structure of catechol

1.6.1.12 Sinapic acid

Sinapic acid (Figure 12) is a common hydroxycinnamic acid which is synthesized in the plant kingdom. Sinapic acid has potential for antioxidant activity (Kikuzaki *et al.*, 2002). It is an allelochemical compound with allelopathic potential on plant physiology (Siqueira *et al.*, 1991; Blum *et al.*, 1999; Lara‐Núñez *et al.*, 2009). Lima *et al.* (2013) investigated the effects of sinapic, p-coumaric, caffeic, ferulic and cinnamic acids on root growth, lignin and the composition of p-hydroxyphenyl, guaiacyl and syringyl lignin monomers of soybean. Sinapic acid decreased guaiacyl and syringyl contents.

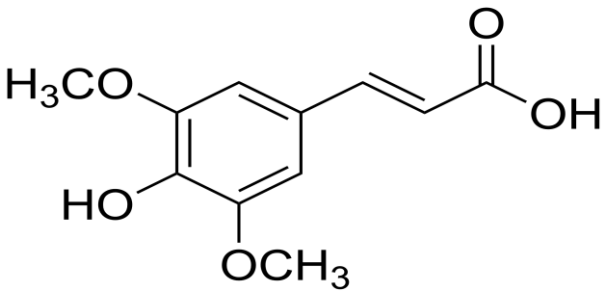


Figure 12 Chemical structure of sinapic acid

1.7 Herbicide trifluralin

Trifluralin (Figure 13) is a selective, pre-emergence herbicide used for eliminating a wide range of weeds (Worthing and Hance, 1991; Senseman and Armbrust, 2007). It was first used as a herbicide in 1963 (Grover *et al.*, 1997). Trifluralin is one of the dinitroaniline group of herbicides, which inhibit mitosis through disrupting cell division and interfere with assembly of microtubules as well as killing germinated seeds at the very early stage (Fernandes *et al.*, 2013).

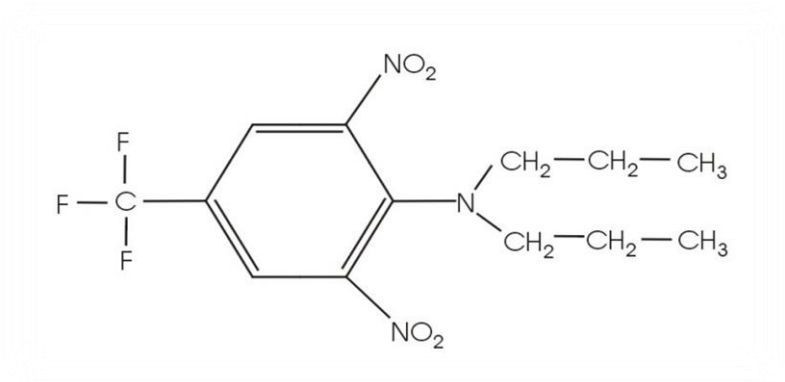


Figure 13 Chemical structure of trifluralin

1.8 Mode of action of allelochemical compounds

Opportunities to use natural phytotoxins in weed management are increased by elucidation of the mode of action of allelochemicals. There are no standard methods for studying their mechanism of action but research teams have studied and developed approaches. For example, analysing allelochemical structures and activity may give clues as to mechanisms of action.

Methods for the identification and quantification of phenolic compounds with allelopathic effects include ion exchange chromatography, capillary electrophoresis, column chromatography on silica, planar chromatography, and counter-current chromatography (Hostettmann *et al.*, 1997).

Potential allelopathic compounds are usually verified by testing their effects on seed germination of susceptible plant species. Inhibition of, or delays to, seed germination of certain species caused by some plants that have allelopathic potential, such as wheat, sunflower, rye, and sorghum, have been reported (Inderjit and Duke, 2003; Weston and Duke, 2003).

Some allelochemicals, including aqueous extracts of sunflower leaves (Bernat *et al.*, 2004a), affect photosynthesis. *Sorghum bicolor* is able to release an allelochemical compound, sorgoleone, a lipophilic benzoquinone that inhibits photosynthesis by influencing photosystem II so weed seed production might be decreased because of the negative impacts from allelochemicals on the photosystem process (Czarnota *et al.*, 2001).

The action of allelochemicals on respiration has also been examined. They may affect mitochondrial respiration and inhibit O_2 uptake. For example, sunflower leaf aqueous extracts inhibit mustard seed germination by minimizing the rate of seed respiration in the first three days of germination. This may be due to the influence of allelochemicals from the sunflower leaves (Bogatek *et al.*, 2005).

Some studies suggest that allelochemicals may reduce plant growth by inhibition of mitosis and mitochondrial activity. When allelochemicals affect root growth, mitosis can be analysed by using onion roots to study the allelopathic impacts on root cell division (Dayan *et al.*, 2000). Mitotic index analysis is one of the methods used for studying allelopathic impacts on plants. This index is a measure of the number of dividing cells within the total number of cells within the zone under observation. *Zea mays* allelochemicals reduced the mitotic activity of radicle meristematic cells of *Citrullus lanatus* (Gniazdowska and Bogatek, 2005).

Certain other metabolic processes and compounds may also be affected in plants that are susceptible to the effects of allelochemicals and these are considered in the following sections.

1.8.1 Sugar content

Sugars are carbohydrate organic compounds which are produced during photosynthesis and play an essential role in providing energy in respiration. Moreover, sucrose can be converted to form starch which is a longer term energy store. Sugars also play a crucial role in the structure of plant cell walls (Harborne, 1998; Stitt and Zeeman, 2012). In addition to these functions, sugars play regulatory roles in many aspects of plant growth and development (Rosa *et al.*, 2009; Stokes *et al.*, 2013; Lastdrager *et al.*, 2014).

There are reports on the importance of sugars in the resistance of plants to diseases caused by fungal pathogens and oomycetes: recently their role as signal molecules in resistance responses has been shown (Morkunas *et al.*, 2011; Moghaddam and Van den Ende, 2012).

A large number of genes are sugar regulated at the transcriptional level, including genes which are involved in carbon and nitrogen metabolism, photosynthesis processes, stress responses, and secondary metabolism in various plant species (Sheen *et al.*, 1999). Chan and Yu (1998) found that sugars can suppress gene expression through affecting mRNA stability by specific 3' untranslated region sequences.

Allelochemicals may exert an effect by influencing sugar metabolism in susceptible plants. Singh and Sunaina (2014) found that application of the highest tested concentration (1.5 mM) of ferulic acid significantly reduced sugar content of tomato. Mohamadi and Rajaie (2009) studied the effects of aqueous eucalyptus (*Eucalyptus camaldulensis*) extracts on seed germination and seedling growth of *Phaseolus vulgaris* and *Sorghum bicolor*. The extracts significantly reduced soluble sugar content in both species. In complete contrast, however, Ahmad and Bano (2013) found that soluble sugar contents were significantly increased by the application of maize extracts to soybean.

1.8.2 Protein content

Proteins are essential in all biological processes (Berg *et al.*, 2002). Measurement of total protein content is common to many applications in research and in clinical laboratory practice. It reflects impacts on protein synthesis and degradation, etc. Preston (2002) mentioned that synthetic herbicides interfere with enzymes or other proteins that eventually influence the growth and metabolism of plant systems and allelochemicals may have similar mechanisms.

Verma and Rao (2006) reported that protein content of different varieties of *Glycine max* was increased in seedlings exposed to weed extracts from *Ageratum conyzoides* and *Solanum nigrum*. Further to the previous studies on protein content, Mali and Kanade (2014) found that aqueous leaf extracts of *Alternanthera sessilis* and *Cynodon dactylon* increased protein content two or three times more than control treatment in sorghum. However, Kaur and Sharma (2015) observed that application of aqueous extracts of *Ageratum conyzoides* significantly decreased protein content of *Vigna radiata*.

1.8.3 Proline content

The amino acid proline (Figure 14) is one of the osmoprotective molecules which protect organisms from stress. According to Yoshiba *et al.* (1997), it is a very common compatible osmolyte in plants. It is capable of accumulating in various organisms such as invertebrates, bacteria, fungi and plants in response to water stress and salinity (Abraham, 2004). Proline has also been recognised as a general stress indicator. Therefore, the content of this amino acid in plants may be indicative of allelopathic effects.

Durán-Serantes *et al.* (2002) reported that allelochemicals (2-benzoxazolinone, p-hydroxybenzoic acid, and ferulic acid) and herbicides (linuron and fluometuron) affected free proline accumulation in *Dactylis glomerata*. The two herbicides almost doubled free proline. Another study by Kamal (2010) found that sunflower allelochemicals significantly increased accumulation of proline in wheat (*Triticum aestivum*). Water extract from sunflower leaves increased free proline more than stem and root water extracts in two varieties of wheat (Margalla 99 and Chakwall 97). Moreover, Das *et al.* (2012) found that aqueous leaf leachates of six tree species (*Acacia auriculiformis*, *Albizia lebbeck* , *Eucalyptus citriodora*, *Emblica officinalis*, *Shorea robusta* and *Tectona grandis*) significantly increased proline content in *Cicer arietinum*.

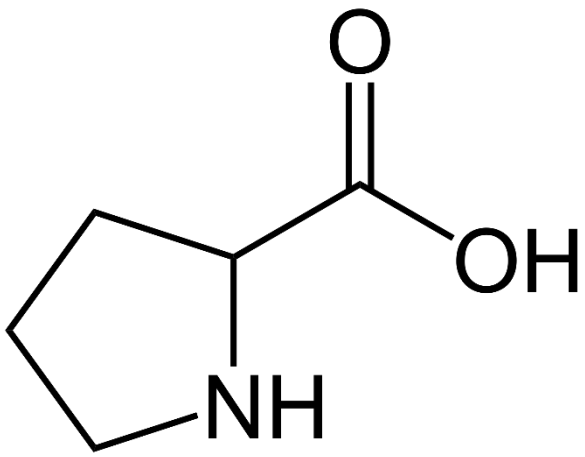


Figure 14 Chemical structure of proline

1.8.4 Total DNA content

Allelochemicals are associated with the inhibition of cell division through effects on mitosis, chromatin organization, and DNA physical and chemical properties (Zhang *et al.*, 2010; Teerarak *et al.*, 2012). When the cell division process is disturbed e.g. during germination of seeds, seedlings grow slowly or die (Imatomi *et al.*, 2013).

Mohamed and El-Ashry (2012) observed that aqueous extract of black mustard (*Brassica nigra*) at 0.25, 0.50 and 1% concentrations significantly inhibited cell division and increased the percentage of chromosomal aberrations in mitotic and meiotic cell divisions of pea (*Pisum sativum*).

Kamal (2010) investigated the effects of sunflower (*Helianthus annuus*) aqueous leaf, stem and root extracts on total DNA content. Total DNA content of wheat (*Triticum aestivum* L.) was significantly increased compared to control treatments. It was also observed that sunflower leaf extract had more effect than root aqueous extract while stem aqueous extract had the least effect on total DNA content.

Padhy *et al.* (2000) studied the effects of different concentrations (5, 10, l5 and 20%) of water leachates of *Eucalyptus globulus* on physiological and biochemical processes of finger millet (*Eleusine corocana*). They found that all concentrations significantly decreased total DNA and RNA contents of shoots and roots.

1.8.5 Plant hormones

Phytohormones are chemical compounds which are produced by plants and can be the main internal factors to control plant growth and development (Hartmann and Kester, 1968; Fosket, 1994). Plant hormones play an essential role in regulation of life cycle events in plants. For instance, plant hormones regulate cell division and extension, seed germination and seed dormancy, flowering and fruiting. Plant hormones occur and are effective at very low concentrations (ng g^{-1}) compared with other chemical compounds (Kelen *et al.*, 2004). The term "hormone" was first mentioned in plant physiology by Fitting (Weyers and Paterson, 2001). He reported that orchid pollinia contain some materials that cause swelling. In 1926, Went worked on isolation of material from coleoptile tips that encouraged cell elongation of coleoptiles: he called this material auxin (Malamy *et al.*, 1990). Moreover, after several studies, indole-3-acetic acid (Figure 16) was identified as a main natural auxin (Taiz and Zeiger, 2002). Higher concentrations of auxin stimulate cell division, which can help photosynthesis (Singh and Gerung, 1982). The regulation by auxin can be changed by the levels of indole acetic acid or depends on the sensitivity of plant tissues (Firn, 1986).

Gibberellins (GA) were first isolated by Kurosawa in 1926 from the fungus *Gibberella fujikoroi* after he observed that when plants were infected with this fungus, their stems elongated (Stowe and Yamaki, 1957). The active material (gibberellic acid) was called gibberellin. Some physiological influences of gibberellins (Figure 15) include stem elongation by stimulating cell division, seed dormancy break and flowering (Raven *et al.*, 2005).

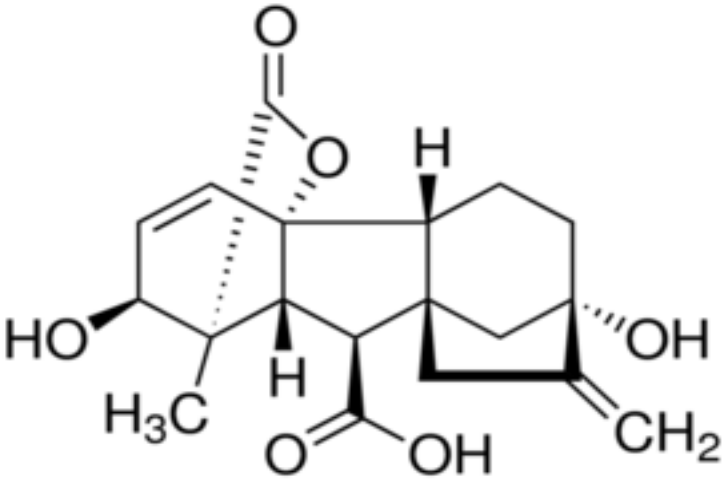


Figure 15 Chemical structure of gibberellic acid

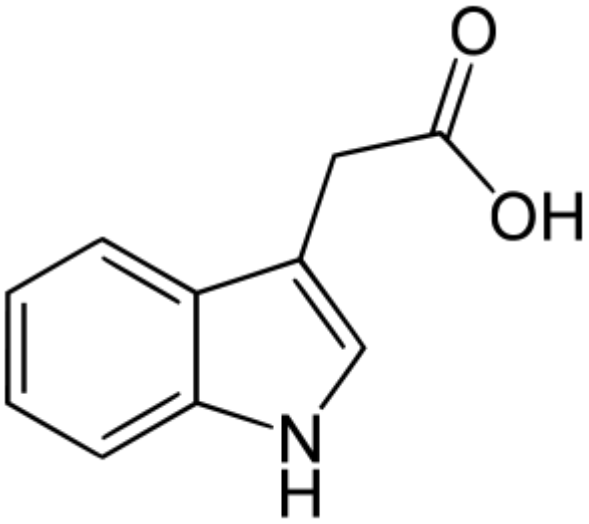


Figure 16 Chemical structure of indole-3-acetic acid

Abscisic acid (ABA) was separated and identified from cotton bolls as biologically active (Liu and Carns, 1961). This phytohormone plays an essential role in many aspects of plant growth, such as seed germination, seed dormancy, and plant stress responses, for example to drought and osmotic stress. ABA (Figure 17) is considered to be a plant growth inhibitor because of the

inhibitory effect of exogenously applied ABA on seed germination and growth when used in bioassays. Neverthless, Sharp *et al.* (2000) showed that endogenous abscisic acid may play an important role in promoting plant growth (Finkelstein *et al.*, 2002). ABA also regulates the final phases of somatic embryo development and embryo quality through enhancing tolerance to desiccation and prevents germination (Rai *et al.*, 2011).

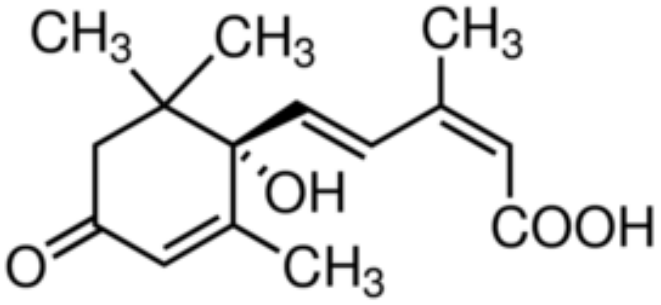


Figure 17 Chemical structure of abscisic acid

Allelochemical effects on plant growth are implicated in production and control of phytohormone levels. This involvement could represent an essential factor affecting regulation of numerous metabolic processes which control plant growth (Olofsdotter, 1998). Secondary metabolites influence phytohormones (gibberellins and auxins) and significantly affect cell elongation in plants.

Kamal (2010) studied allelopathic effects of sunflower *(Helianthus annus* L.) on plant hormone contents (indole acetic acid, gibberellic acid and abscisic acid) of two wheat varieties (Margalla 99 and Chakwall 97) in a petri dish experiment. Sunflower aqueous extracts (leaves, stems, and roots) significantly decreased indole acetic acid and gibberellic acid. However, abscisic acid content in wheat seedlings was significantly increased.

Another study, by Kefeli and Turetskaya (1968), showed the effect of allelochemicals on plant hormone activity. The study indicated that some phenolic compounds extracted from aqueous extracts of some weed plants have inhibitory effects on the activity of IAA and gibberellin (GA).

Balah and Latif (2013) studied the influence of aqueous extracts of medicinal plants *Thymus vulgaris, Salvia officinalis* and *Calendula officinalis* on plant hormonal content of wheat (*Triticum aestivum* L.) and its associated weeds *Lolium multiflorum* and *Phalaris paradoxa* under laboratory conditions. Aqueous extracts of *Thymus vulgaris* reduced IAA content of wheat seedlings but GA and ABA content were reduced by *Calendula officinalis* extracts.

1.8.6 Chlorophyll content

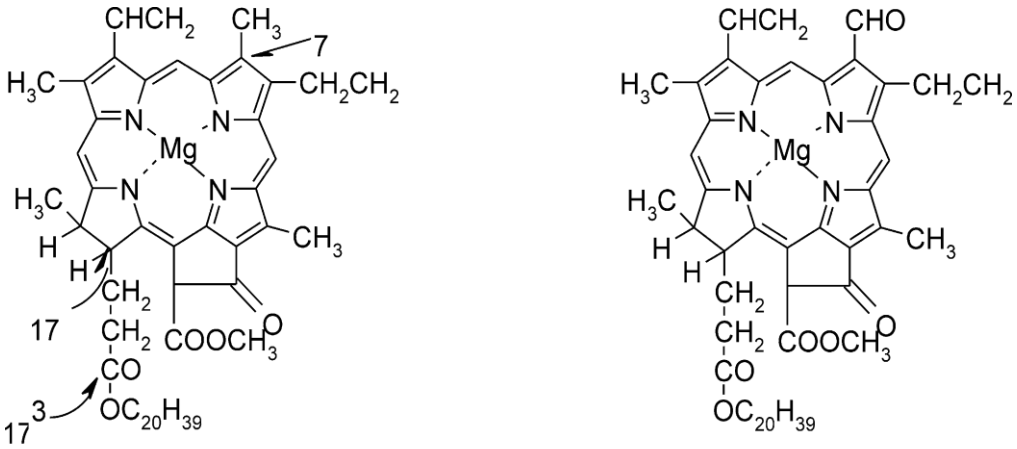
The term chlorophyll (Figure 18) is a Greek hybrid word, from "Chloros", which means "green", and "phyllos", which means "leaves". It was first mentioned in 1818 in relation to pigments extracted from plant leaves using organic solvents (Scheer, 2006). Chlorophyll was identified by using spectroscopy and techniques of solvent partition (Stokes, 1863). Chlorophylls are the main drivers of the photosynthesis processes which absorb light and transfer energy (Liu *et al.*, 2012). The efficiency of photosynthesis is based on the concentrations of chlorophyll in plant tissues (Chen *et al.*, 2013). Chlorophylls absorb light energy and they transfer light energy to excitation energy as well as with high quantum efficiency to the reaction centre.

It has been reported that phenolic acids have allelopathic inhibitory effects on plant growth via influencing photosynthesis and chlorophyll content (Einhellig, 1995). Yang *et al.* (2004) investigated the effects of three phenolic acids (o-hydroxyphenylacetic, ferulic and p-coumaric acid), at concentrations of 50, 100 or 200 ppm, on chlorophyll accumulation of leaves of rice (*Oryza sativa*) in a greenhouse experiment. Chlorophyll content was decreased by the application of all studied phenolic acids. Ferulic acid at the highest concentration had the greatest inhibitory effect while o-hydroxyphenylacetic and p-coumaric acids had greater inhibitory effects at concentrations of 50 and 100 ppm. Kamal and Bano (2009) showed the effect of sunflower leaf, stem and root extracts on chlorophyll accumulation in two varieties of wheat seedlings (Margalla 99 and Chakawall 97) in a petri dish experiment. Sunflower leaf aqueous extract at a concentration of 1 g /9 ml distilled water significantly reduced chlorophyll content in both varieties, followed by stem and root extract.

Farhoudi *et al.* (2015) also examined effects of sunflower shoot aqueous extract on seedling growth, photosynthesis and activities of enzymes of two weed species - johnson grass (*Sorghum halepense*) and wild mustard (*Sinapis arvensis*). Sunflower crude extract at higher concentrations (30%) reduced photosynthesis and chlorophyll a and b accumulation in johnson grass seedling leaves compared with the control treatment.

Furthermore, Benyas *et al.* (2010) studied the effect of aqueous extract of cocklebur (*Xanthium strumarium*) at different concentrations (0, 0.5, 1, 1.5 and 2% w/v) on seed germination, growth and chlorophyll accumulation in lentil (*Lens culinaris*) in a greenhouse experiment. Shoot aqueous extract of *Xanthium strumarium* at low concentrations had no significant effect on germination, growth, total chlorophyll, chlorophyll a and chlorophyll b content. Nevertheless, higher concentrations significantly affected seed germination, shoot and root length, and dry weight.

Elisante *et al.* (2013) also studied the influences of jimsonweed (*Datura stramonium*) leaf and seed aqueous extract at different concentrations (0%, 25%, 50%, 75% and 100%) on seedling growth and chlorophyll content of buffel grass (*Cenchrus ciliaris*) and glycine (*Neonotonia wightii*) in pot experiment conditions. Higher concentrations of aqueous seed and leaf extracts of *D. stramonium* reduced total chlorophyll content, shoot and root length and fresh and dry weight of *C. ciliaris* and *N. wightii*.



Chlorophyll *a* Chlorophyll b

Figure 18 Chemical structures of chlorophylls a and b (Schoefs, 2002)

1.9 Study aims and objectives

The major aim of this study was to determine the allelopathic potential of sunflower shoot and root extracts to affect seed germination and seedling growth of some crop and weed species. Based on sunflower growth stages, the study also focused on identification and determination of phenolic compounds and examination of their effects on germination and growth of studied species.

To understand the mechanism of action of allelochemicals, the allelopathic effects of phenolic compounds and trifluralin on sugar, protein, proline, DNA, GA, IAA and ABA contents of *Brassica napus*, *Cephalaria syriaca*, *Avena fatua* and *Triticum aestivum* were also evaluated.

The major objectives of this study were to:

1. Evaluate the allelopathic effects of sunflower shoot and root aqueous extracts from sunflower plants of different ages/growth stages (one week, one month, two month and mature stage) on seed germination and seedling growth of some crop and weed species. Allelopathic potential may change with age/growth and there may be an optimum. Identification of this would be important in developing effective bioherbicides based on sunflower extracts.

2. Identify and quantify phenolic acids in sunflower shoot and root aqueous extracts by using high performance liquid chromatography (HPLC).

3. Investigate the chemical composition of exudates to identify the key chemical compounds responsible for the allelopathic effects.

4. Evaluate the herbicidal effects of these 'isolated' chemicals individually and in combination and in comparison with sunflower extracts/residues and a typical synthetic herbicide.

5. Elucidate the mechanism of action of total phenolic compounds, trifluralin and sunflower shoot aqueous extract on germination and growth of some crop and weed species.

6. Compare the effects of sunflower two month shoot aqueous extract and soil incorporated sunflower ground shoot on germination and growth of some crop and weed species.

CHAPTER 2

MATERIALS AND METHODS

2.1 Effects of sunflower aqueous shoot and root extracts from plants at different growth stages (one week, one month, two month and mature stage)

2.1.1 Sample collection

Sunflower plants, var. Coupon, were sown in April 2013 and grown based on standard agricultural conditions at the farm of the Agricultural Institute, Erbil, Kurdistan Region, Iraq. Plants were collected at three growth stages: one month, two months and mature stage. Samples of plants from each of the three different growth stages were separated into the shoot and root fractions. Roots and shoots were chopped into 5 cm pieces and then air dried for about two weeks. When the samples were completely dry, they were sent to Newcastle University where they were ground into fine particles with a bench-mounted hammer mill. As there were no 'one week', i.e. less than one month old, sunflower plants available from Kurdistan region, plants were grown from seed at Newcastle University. Seeds were germinated at 25 °C in the dark in a growth chamber (Sanyo, model MLR-351) and harvested after seven days. The sunflower seedlings were then separated into the shoot and root fractions, air dried for two weeks and ground with a coffee grinder.

2.1.2 Preparation of aqueous extract of dried sunflower shoots

Aqueous extracts of sunflower (shoot and root) from plants of different growth stages (one week, one month, two month and mature stage) were prepared by mixing 10 g sunflower shoot and root separately with 100 ml distilled water and shaking overnight. The extracts were filtered, and then centrifuged at 1000 rpm for 10 min. The supernatant was filtered through a micro pore filter (0.45 µm). The resultant extracts were stored at 4 °C until required for the germination tests.

2.1.3 Laboratory seed germination experiments

Seeds of rye (*Secale cereale*), wheat (*Triticum aestivum*), wild oat (*Avena fatua*), pea (*Pisum sativum*), Syrian Cephalaria **(***Cephalaria syriaca*), little seed canary grass (*Phalaris minor*), rapeseed (*Brassica napus*) and sunflower (*Helianthus annuus* L.) were used. The crop seeds were obtained from Nafferton Farm, Newcastle University. The weed seeds were purchased from Herbiseed. Prior to the germination test, seeds were sterilized by washing in 10% household bleach solution (sodium hypochlorite) for 15 min followed by rinsing three times in distilled water. This was done to prevent contamination of the germinating seedlings with pathogens borne on the seeds' surface.

2.1.4 Bioassay

Ten seeds of each species were placed in 9 cm diameter petri dishes lined with filter paper. Five ml of four different sunflower shoot and root extracts (one week, one month, two month and mature stage) were added to the petri dishes of all seed samples – these were the treatment or test petri dishes. The control petri dishes received 5 ml distilled water only. There were four replicates of each seed species in both test and control treatments. Seeds were germinated at 25 °C in the dark in a growth chamber. The numbers of germinated seeds were recorded after seven days of incubation to obtain the percentage of germination of the seed samples. Shoot and root lengths and dry weights of the germinated seedlings were also measured (Figure 20). Oven dry weights of seedling shoots and roots were determined after drying samples overnight in an oven at 60 °C.

2.2 Effects of different concentrations of sunflower shoot aqueous extract (mature stage) on *Brassica napus, Secale cereale, Cephalaria syriaca* and *Phalaris minor* seeds

Seeds (10 seeds/petri dish) of *B. napus, S. cereale, C. syriaca* and *P. minor* were placed in petri dishes lined with filter paper. Six different concentrations (0.31%, 0.63%, 1.25%, 2.5%, 5% and 10%) of sunflower shoot water extract (5 ml) were added to the petri dishes. For the control petri dishes, distilled water was used. There were three replicates of each species in both test (different concentrations) and control. Petri dishes were placed into a growth chamber for incubation (25 °C). After 7 days, germination percentage, shoot and root length, and dry weight of germinated seedlings were recorded.

2.3 Freeze drying of sunflower shoot and root aqueous extracts

Sunflower shoot and root aqueous extracts from plants at different growth stages were freeze-dried. Ten grams of each extract (one week, one month, two month and mature stage) were mixed with 100 ml distilled water and shaken overnight. The extracts were filtered, and then centrifuged at 1000 rpm for 10 min. The supernatant was filtered through a micro-pore filter (0.45 μm) membrane. The supernatant was filtered through one layer of Whatman No.1 filter paper. The resultant extracts were freeze-dried. Freeze-dried extracts were weighed to obtain yield of each sample.

2.4. Phytochemical screening of sunflower shoot extracts (mature stage)

2.4.1 Alkaloid extraction from dried sunflower shoots

Five grams of dried sunflower shoot was put into a flask and 200 ml of acetic acid (20% v/v) in ethanol was added. Then the extract was covered and left to stand for four hours. The extract was filtered and concentrated to one-quarter of the original volume by using a water bath. Concentrated ammonium hydroxide was added drop wise to the extract until precipitation was completed. The whole solution was allowed to settle and precipitate was collected by filtration and weighed. Mayer's reagent was purchased from Sigma-Aldrich Ltd. and added to detect alkaloids (Obadoni and Ochuko, 2002; Okwu and Josiah, 2006).

2.4.2 Extraction of phenolic compounds

500 mg of freeze-dried sunflower shoot (mature stage) was extracted in 5 ml of methanol (70 % v/v) HPLC grade (Fisher Chemical, UK). The extract was shaken for 15 min at room temperature and centrifuged at 1000 rpm for 5 min. The supernatant was filtered through Fisher brand QL125 90 mm filter paper. The pellet was re-extracted with 5 ml of 70% methanol and rinsed with 5 ml 100% methanol. All three supernatants were pooled together before removal of the methanol under vacuum with a rotary evaporator at 70 °C. The extract was concentrated and purified by using an activated Sep Pak C18 column (Sep-Pak RC Cartridge, Waters) and eluted with 4 ml of 100% HPLC grade methanol (Rispail *et al.*, 2005). Phytochemical qualitative analysis was carried out according to the method used by Ayeni and Yahaya (2010).

2.4.3 Tannins

0.5 g of ground sunflower shoot was boiled with 20 ml of deionized water in a test tube. After filtration 0.1% of $FeCl_3$ was added to the filtered extract. If the samples were brownish green or blue black this indicated the presence of tannins (Ayeni and Yahaya, 2010).

2.4.4 Phlobatannins

10 ml of sunflower shoot aqueous (section 2.1.2) extract was boiled with a few drops of 1% HCl using a test tube. After that, if a red precipitate was observed then it indicated the presence of phlobatannins (Ayeni and Yahaya, 2010).

2.4.5 Saponins

2 g of powdered sunflower shoot extract (section 2.1.2) was boiled in 20 ml of deionized water in a water bath and then the sample was filtered by using filter paper (Whatman No.1). 10 ml of the filtered extract was mixed with 5 ml of deionized water in a test tube and shaken vigorously in order to obtain a stable persistent froth. The frothing was mixed with three drops of olive oil for the formation of an emulsion which is the indication of presence of saponins (Ayeni and Yahaya, 2010).

2.4.6 Flavonoids

Sunflower shoot aqueous extract (section 2.1.2) was mixed with a few drops of 1% NH_3 solution in a test tube. Flavonoids were present if a yellow coloration appeared (Ayeni and Yahaya, 2010).

2.4.7 Terpenoids

5 ml of sunflower shoot aqueous extract (section 2.1.2) was mixed with 2 ml of $CHCl_3$ in a test tube. 3 ml of concentrated H_2SO_4 was gently added to the mixture to make a layer. Terpenoids were present when an interface with a reddish brown coloration was made (Ayeni and Yahaya, 2010).

2.4.8 Estimation of total phenolics

Phenolic content was determined by using the Folin-Ciocalteu method. Gallic acid was used as standard. 10 g of dry gallic acid was dissolved in 100 ml of distilled water. 7.5 g of sodium carbonate was dissolved in 100 ml deionized water. For making the calibration curve, eight dilutions were made up of the stock solution (0.31%, 0.63%, 1.25%, 2.5%, 5% and 10%). 20 µL of each calibration solution, sample or blank were added to separate cuvettes. Then 1.58 ml distilled water was added to each of the cuvettes and then 100 µL of Folin-Ciocalteu reagent was added. After mixing them well and waiting between 8 s and 8 min, 300 µL of 20% sodium carbonate solution was added. After 2 h at 20 ^{o}C, the absorbance of each solution was determined at 765 nm against the blank by using a spectrophotometer and absorbance was plotted against concentration (Figure 19) (Zhang *et al.*, 2007).

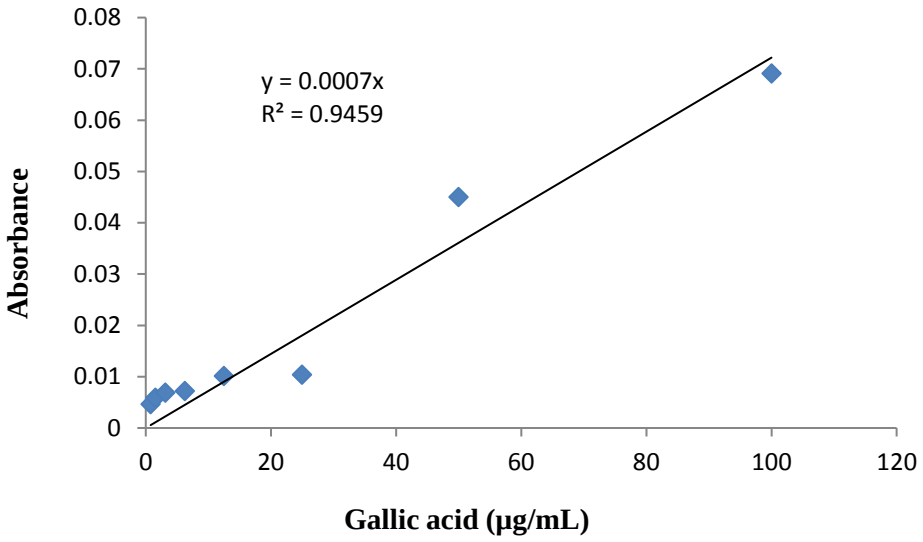


Figure 19 Total phenolic acid standard curve

2.5 Effect of sunflower aqueous shoot extract on mitotic index

Ten seeds of wild oat (*Avena fatua*) were placed in 9 cm diameter petri dishes lined with filter paper. Five ml of sunflower two month old aqueous shoot extract 1:10 (w/v) was added to each petri dish. The control petri dishes received 5 ml distilled water only. There were four replicates of each seed species in both test and control treatments. Seeds were germinated at 25 °C in the dark in a growth chamber. The numbers of germinated seeds were recorded after seven days.

One cm of sample root meristems of *A. fatua* tips were fixed for 24 h with acetic acid, chloroform, and ethanol (6:3:1) with trace iron. The samples were then stored at -20 ºC for 3 days before analysis. Wild oat root tips were hydrolysed with hot 1 N HCl for about 25 min at 60 ºC to achieve dispersion of cells and chromosomes. The samples were then stained with Schiff´s reagent (Sigma-Aldrich). Meristems were embedded in a drop of acetic acid, then cut on a slide, and heated in a flame. After covering with a coverslip, the wild oat meristems were squashed and heated again. After sample preparation, meristems were scored by light microscopy using the x40 objective lens and the mitotic indices were estimated on a total of 300 cells of each root sample in three slides of the same sample (Martínez *et al.*, 2003).

Mitotic Index Formula

$$\text{Mitotic Index} = \frac{P+M+A+T}{\text{Total number of cells scored}} \times 100$$

Where:

P = Prophase, M = Metaphase, A = Anaphase, T = Telophase

2.6 Effect of sunflower aqueous shoot extract on cell elongation

Wild oat root tips for cell elongation measurement were used after seven days seed germination as described in section 2.5. After germination, meristems were taken from roots of control and test wild oat seedlings and then prepared for measurement by light microscopy using the x40 objective lens and cell lengths were estimated on a total of 20 cells of each root sample in three slides of the same sample.

2.7 Pot experiments

2.7.1 Sample collection

Sunflower plants were collected as described in **2.1.1** above.

2.7.2 Incorporation of two month old sunflower ground shoot in soil-pots (growth chamber experiment)

This experiment was performed in a growth chamber. Ground sunflower plant material was incorporated into the soil at different concentrations (3, 6 and 9 g/pot) with no addition of the ground shoots into the control treatments. Rapeseed (*Brassica napus*), wild oat (*Avena fatua*), Syrian Cephalaria (*Cephalaria syriaca*) and littleseed canary grass (*Phalaris minor*) were seeded in topsoil which was purchased from Wickes company (500 g soil each pot). The growth chamber condition was maintained at 24/16 ºC day/night, with light 16/8 h (day/night) and natural humidity. Seven seeds of the selected weed species were sown and watered when required. Each treatment had three replications (Nektarios *et al.*, 2005; Rajput and Rao, 2013).

2.7.3 Application of two month old sunflower aqueous shoot extract to seeds in pot experiment (growth chamber experiment)

This experiment was conducted in a growth chamber. In this experiment, different concentrations (0%, 3%, 6% and 9%) in ratio 1:10 (w:v) of aqueous two months shoot extract were applied to rapeseed (*Brassica napus*), Syrian Cephalaria (*Cephalaria syriaca*), wild oat (*Avena fatua*) and littleseed canary grass (*Phalaris minor)* in soil. The growth chamber was maintained at 24/16 ºC day/night, with light 16/8 h (day/night) and natural humidity. Each pot

was filled with 500 g of topsoil. Each pot was seeded with seven seeds of all seed species. Pots were supplied with 100 ml of the sunflower shoot aqueous extract and for the control treatment, distilled water was applied (Naderi and Bijanzadeh, 2012).

After two weeks, the following parameters were measured for both ground shoots and aqueous extract experiments:

2.7.4 Seed germination (%)

2.7.5 Shoot and root length (cm/plant)

A ruler was used for measuring shoot and root length. The measurement of shoot length was from the base to the top of the shoot system. Root length was measured from the base to the end of root system (Figure 20).

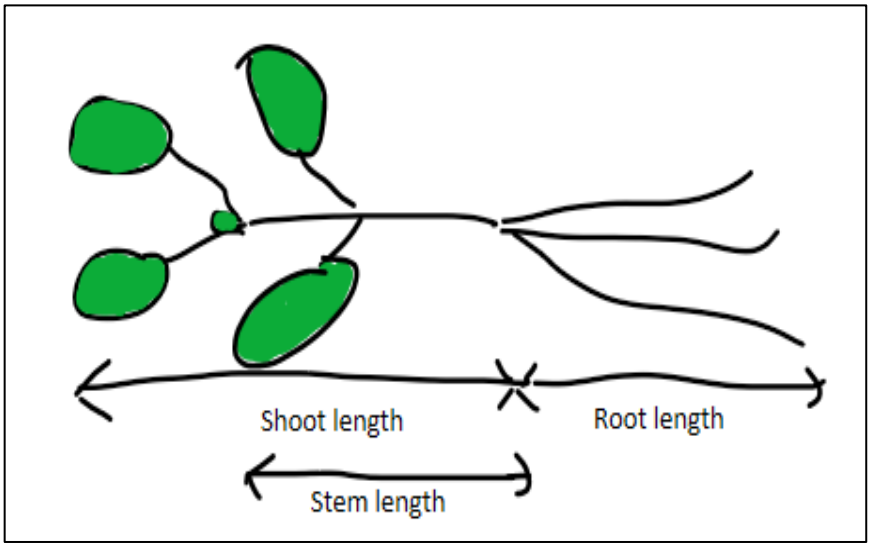


Figure 20 Shoot and root length measurement method

2.7.6 Shoot and root dry weight (mg/plant)

2.7.7 Soil mineral content

After two weeks of experiment and harvesting plants, soil samples were obtained from each pot. After sampling, soil was air dried and then sieved with a 0.5 cm sieve. Macro minerals (K, Ca and Mg) were analysed by using Palintest extraction method kit (SK400).

2.7.8 Soil pH and EC (Electrical Conductivity)

pH and EC were assessed as follows: 10 g of topsoil samples were mixed with 25 ml distilled water and stirred thoroughly with a stirring rod for about one minute. After 10 minutes, samples were stirred again. pH of soil samples was measured by immersing the electrode in the supernatant part of samples. EC of soil samples was measured by immersing the calibrated electrode in the soil samples.

2.7.9 Chlorophyll content

2.7.9.1 Sample preparation method

A modified method based on Rashed (2009) was used. 70 mg of freeze-dried leaf samples were weighed and placed in 10 ml screw glass tubes, and then 1 ml ethyl acetate was added. Samples were covered with aluminium foil in order to avoid light. Samples were vortexed for a few minutes and put into the fridge overnight. Samples were then centrifuged for 10 minutes (4000 rpm speed). Supernatants were put into 10 ml screw glass tubes with a Pasteur pipette. Samples were sealed with standard HPLC vial caps for HPLC analysis.

2.7.9.2 HPLC analysis for chlorophyll content

Chlorophyll a and chlorophyll b analysis was carried out by using HPLC (Shimadzu Corporation. Kyoto, Japan). A Hyper Clone Reverse phase C18 (250 × 4.6 mm, 5 µm) column was used, and column oven set at 40 °C. The mobile phase was deionized water solvent (A) methanol solvent (B), and ethyl acetate solvent (C). The gradient of solvents (A, B, C) was: 0 min (50:50:0), 6 min (50:50:0), 11 min (30:70:0), 30 min (15:85:0), 35 min (0:100:0), 38 min (0:90:10), 56 min (0:60:40), 62 min (0:0:100), 64 min (0:0:100), 70 min (0:100:0), 75 min (50:50:0) and 95 min (50:50:0). The flow rate was 1 ml/min, and injection volume 20 µl: the detection was at 450 nm for chlorophyll a and chlorophyll b.

2.8 Effect of phenolic compounds on germination and early growth of *Brassica napus, Cephalaria syriaca, Triticum aestivum* and *Secale cereale*

2.8.1 Identification and quantification of phenolic compounds in sunflower shoot and root extract

2.8.1.1 Sample preparation

40 mg freeze dried ground sunflower shoot and root extracts (one week, one month, two month and mature stage) were weighed into 10 ml screw-glass tubes. The freeze-dried samples were then put into a heating block at 50 ℃. To each sample 950 µL of 70% (v/v) HPLC-grade methanol in deionized water was added. The tubes of samples were sealed by using tube caps and vortex mixed for 20 min at 70 ℃ with vortexing the samples every 5 min for optimization. The samples were centrifuged (4000 *g*, 4 ℃, 20 min). 600 µL of the supernatant was transferred to a new micro tube. Then each sample was taken up into a 1 ml syringe and then filtered (0.2 µm) into a screw top HPLC vial prior to HPLC analysis (Table 1). Twelve phenolic compounds were purchased from Sigma-Aldrich and used as standards to identify and quantify the compounds from sunflower extracts.

Phenolic compound calculation:

An external standard method was used to calculate the amounts of phenolic compounds from sunflower extracts by using HPLC.

$$\text{Response Factor of Standard} = \frac{\text{Standard Peak Area}}{\text{Standard Concentration}}$$

$$\text{Sample Concentration} = \frac{\text{Sample Peak Area}}{\text{Response Factor of Standard}}$$

Table 1 HPLC method for phenolic acid analysis.

Column	C18 (250 × 4.6 mm, 5 μm)
Oven temperature	25 ℃
Wavelength	260, 280 and 320 nm
Flow rate	1 mL/min
Injection volume	20 μL
HPLC brand	Shimadzu Corporation. Kyoto, Japan
Mobile phase	0.1% v/v trifluoroacetic acid in deionized water (solvent A) and 0.1% v/v trifluoroacetic acid in acetonitrile (solvent B)
Solvent gradient	0 min (100:0), 5 min (100:0), 15 min (83:17), 17 min (83:17), 22 min (75:25), 30 min (65:35), 35 min (50:50), 40 min (0:100), 50 min (0:100), 55 min (100:0) and 65 min (100:0).

2.8.2 Effect of phenolic compounds on seed germination and early growth of *Brassica napus, Cephalaria syriaca Triticum aestivum* and *Secale cereale*

2.8.2.1 Laboratory seed germination experiments

Phenolic compounds (gallic acid, syringic acid, vanillic acid, protocatechuic acid, catechol, 4-hydroxybenzoic acid, p-coumaric acid, sinapic acid, ferulic acid, caffeic acid, chlorogenic acid, and trans-cinnamic acid) were purchased from Sigma-Aldrich. Seeds of *B. napus*, *C. syriaca*, *T. aestivum* and *S. cereale* were used. After sterilization by washing in 10% household bleach solution, ten seeds were placed in each petri dish lined with filter paper. Five millilitres of each phenolic compound individually and all twelve together were added to petri dishes at the concentrations specified in Table 5. The concentrations of phenolic compounds were chosen based on their concentrations that had been measured in sunflower extracts by HPLC. The total phenolic compounds combination was prepared by mixing all twelve compounds at the concentrations shown in Table 5. For the control petri dishes, distilled water was used. There were three replicate dishes of each seed species in both tests and controls. Petri dishes were

placed into a growth chamber for incubation (25 °C). After seven days of germination the following parameters were examined

- Seed germination (%).

- Shoot and root length (cm/plant).

- Shoot and root oven dry weight (mg/plant).

2.9 Effect of the herbicide trifluralin on seed germination and seedling growth of *Brassica napus* and *Cephalaria syriaca*

Trifluralin was purchased from Sigma-Aldrich. Ten seeds of *B. napus* or *C. syriaca* were placed in a petri dish lined with filter paper. Seeds were sterilized using the standard method described previously. Ten different concentrations (0, 12.5, 25, 50, 75, 100, 125, 150, 300, 600, 900 ppm) of trifluralin (5 ml) were added to the petri dishes. For the control petri dishes, distilled water was used. There were three replicate dishes of each seed species in both test and control treatments. Petri dishes were placed into a growth chamber for incubation (25 °C). Seed percentage seed germination and seedling growth were measured after seven days.

2.10 Investigations on the mechanism of herbicidal action of aqueous extracts of sunflower shoots, their total phenolic compounds and the herbicide trifluralin on seed germination and early growth of some weed and crop species

2.10.1 Seed germination

Three treatments (total phenolic compounds, trifluralin and two month old sunflower aqueous shoot extract) were applied to *Brassica napus, Cephalaria syriaca, Triticum aestivum,* and *Secale cereale,* in a germination test consisting of seeds incubated in petri dishes.

Seeds were sterilized using the standard method described previously. For each treatment, three different replicates were evaluated. Each replicate included 10 seeds placed in a 10 cm diameter plastic petri dish lined with filter paper (Whatman No.1).

Five millilitres of total phenolic compound mixture shown in Table 5 (combined concentration 0.27 mg/ml), trifluralin (100 ppm) and extracts of two month old sunflower aqueous shoot extract (3% v/v) were added to the petri dishes. The control petri dishes received 5 ml distilled

water only. Seeds were germinated at 25 °C in the dark in a growth chamber. The number of germinated seeds was recorded after seven days germination to obtain the percentage of germination of the seed samples.

After one week of germination, the following parameters were studied.

2.10.2 Sugar content

Sugar content was evaluated by the method of Hodge and Hofreiter (1962). About 0.25 g of fresh plant leaves was homogenized in 2.5 ml of 95% ethanol by using a mortar and pestle. The samples were centrifuged (4000 *g*, 4 °C, for 20 min) and the sugar content was estimated in 0.1 ml of the supernatant. Anthrone reagent was purchased from Sigma-Aldrich. About 4 ml of anthrone reagent was mixed with the sample and heated in a water bath for 8 min. The absorbance of samples using a spectrophotometer was recorded at 620 nm after a rapid cooling. Glucose was used to make a standard curve (Figure 21).

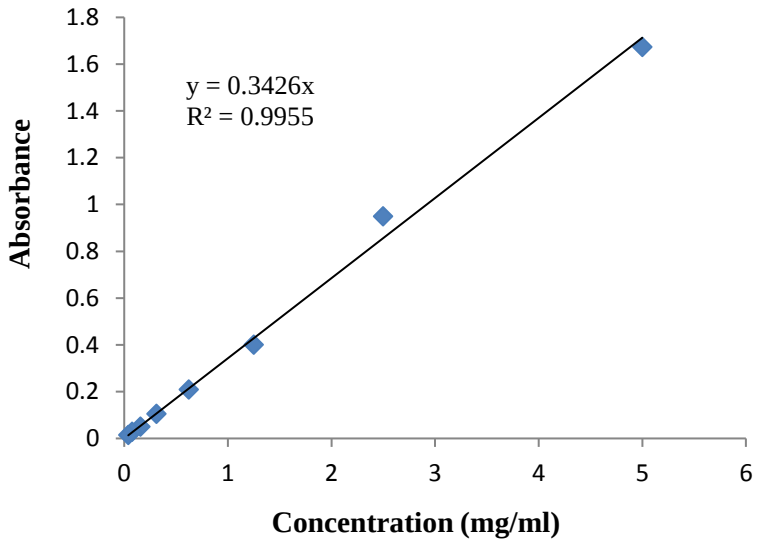


Figure 21 Standard curve for sugar content

2.10.3 Protein content

Protein content was estimated by the Bradford method (Bradford, 1976). About 200 mg of fresh plant leaves was ground in liquid nitrogen using a cooled mortar and pestle with 0.05 g polyvinylpolypyrrolidone (Sigma-Aldrich). After that the powder of each sample was resuspended in 1 ml Tris buffer which contains 0.05 M Tris base, 0.1% (w/v) ascorbic acid, 0.1% (w/v) cysteine hydrochloride, 1% (w/v) polyethylene glycol 4000, 0.15% (w/v) citric acid, and 0.008% (v/v) 2-mercaptoethanol (Arulsekar and Parfitt, 1986). After resuspension, samples were centrifuged (19000 *g*) at 4 °C for 20 min. 0.1 ml of supernatant was mixed for protein dye-binding reaction with 3 ml of Bradford reagent containing 0.01% Coomassie Brilliant Blue G-250, 4.7% (v/v) ethanol (97%) and 8.5% (v/v) phosphoric acid (85%). Absorbance was recorded at 595 nm after 5 min for quantification of the protein content. Bovine serum albumin (BSA) was used as standard (Figure 22).

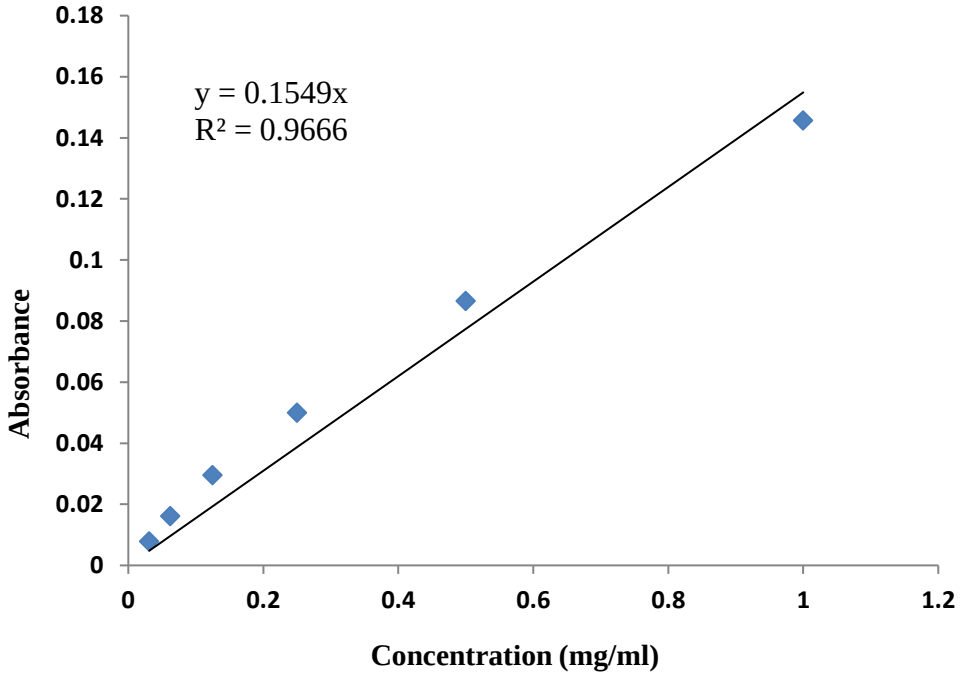


Figure 22 Standard curve for protein content

2.10.4 Proline content

Proline content was estimated from leaves based on the method used by Carillo and Gibon (2011). 50 mg fresh weight aliquots of sample were mixed with 1 ml of ethanol: water (40:60 v/v). The resulting mixture was left overnight at 4 °C. The mixture was then centrifuged at 14000 *g* for 5 min. The process was repeated on the pellet and then supernatants were used for doing analyses as the first extraction recovers more than 93% (Carillo *et al.*, 2008).

Fresh extract was diluted 20 times (w/v) in a 70:30 (v/v) ethanol: water mixture (Hummel *et al.*, 2010). Proline was purchased from Sigma-Aldrich and concentrations from 0.0035 to 1 mM were made for obtaining the standard curve using the same medium as used for extraction.

In 1.5 ml tubes, 1 ml of reaction mix (ninhydrin 1% w/v in acetic acid 60% v/v, ethanol 20% v/v) and 0.5 ml ethanolic extract were added. For standards (1-0.5-0.225-0.125-0.062-0.031-0.015-0.007-0.0035 mM), 0.1 ml of each proline standard solution was pipetted and diluted with 0.4 ml of ethanol: water (40:60 v/v). The tubes were sealed and the mix heated at 95 °C using a block heater for 20 min. Samples were then centrifuged at 1000 rpm for 1 min and the tube contents were transferred to 1.5 ml cuvettes. The absorbance of samples was recorded by using a spectrophotometer at wavelength 520 nm (Figure 23).

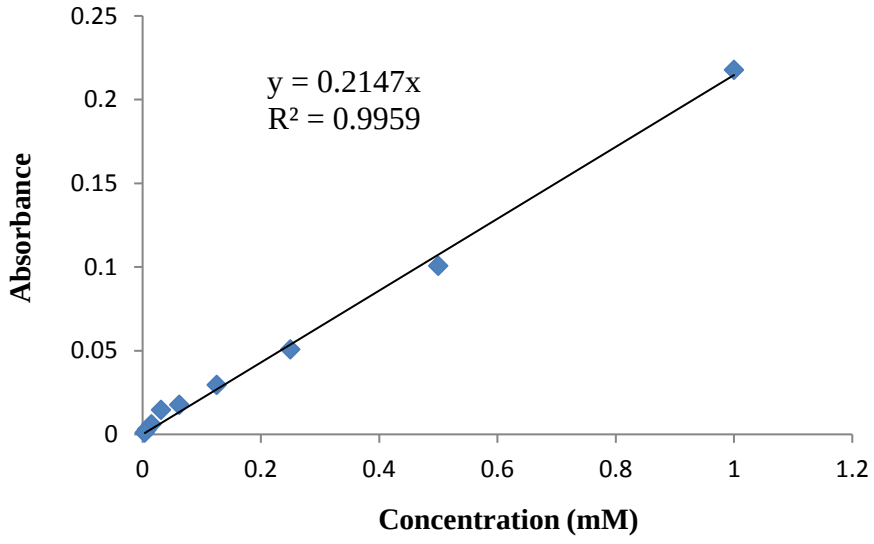


Figure 23 Standard curve for proline content

2.10.5 Total DNA content

Total DNA content was determined from frozen root tissues of samples by using a Nano Drop (ND-1000) spectrophotometer. A DNA isolation kit (DNeasy Plant Mini Kit) was purchased from Qiagen Company. Based on the kit 100 mg of frozen root was extracted in liquid nitrogen and then the kit instruction manual was followed.

2.10.6 Plant hormone contents

2.10.6.1 Sample preparation

Fresh plant leaves were ground in 80% (v/v) methanol mixed with antioxidant BHT (butylated hydroxytoluene) (1 µg/100ml) then kept for 3 days in a refrigerator with solvent being changed each day. The samples were centrifuged and the supernatant was reduced to the aqueous phase through using a rotary thin film evaporator: the pH of the aqueous phase was adjusted to 2.5 to 3.0. The aqueous phase was partitioned 4 times with 1/3rd volume of ethyl acetate. A rotary thin-film evaporator was used to evaporate ethyl acetate. 1 ml methanol (100%) was added to the dried sample and transferred into a screw top HPLC vial prior to analysis (Kamal and Bano, 2008).

2.10.6.2 HPLC Method Analysis

Plant hormones were analysed according to the method used by Kamal and Bano (2008). Pure plant hormones (IAA, GA and ABA) were purchased from Sigma-Aldrich and prepared as standards for identification and quantification of the hormones. These phytohormones were identified based on retention time and peak area of the standards, which were investigated with a photodiode array detector (DAD). Methanol, acetic acid, and water in ratio (30:1:70) was used as the mobile phase. The mobile phase was isocratic. The wavelength used for IAA was 280 nm (Sarwar *et al.*, 1992), but for GA and ABA was 254 nm (Jinchang *et al.*, 1994). The samples were injected onto a C18 column (250 × 4.6 mm, 5 µm) and the flow rate was 0.8 ml/min.

2.11 Effect of sunflower ground shoots, their total phenolic compounds and the herbicide trifluralin on seed germination and early growth of some weed and crop species in pot experiment

This experiment was performed in a growth chamber. *Brassica napus*, *Cephalaria syriaca*, *Triticum aestivum* and *Secale cereale* were seeded in topsoil (500 g soil each pot). In this experiment, two month old sunflower ground shoots (9 g/pot), total phenolic compounds (15 ml/pot of 26.5 mg/100ml distilled water; Table 5), and trifluralin (13 ml/pot based on 2.5 litre of 480 g/l active ingredient per hectare) were added to top soil. For the control treatment, distilled water was applied. After the application, 7 seeds of each species were sown and watered when required. Each treatment had three replicates.

After two weeks, the following parameters were studied:

- Seed germination (%).

- Shoot and root length (cm/plant).

- Shoot and root dry weight (mg/plant).

2.12 Statistical analysis

Results of experiments were analyzed using ANOVA general linear model (Minitab software, version 17) for a completely randomized design with a minimum of three replicates. If data were not normally distributed the Log 10 data transformation was carried out before analyzing data. Means of seed germination data were transformed to Arcsin before doing analysis. Significant differences between means were calculated based on Tukey's test ($P \leq 0.05$).

CHAPTER 3 RESULTS

Experiment 1

3.1 Effects of aqueous shoot and root extracts from the different growth stages of sunflower plants on germination and early growth of *Brassica napus, Secale cereale, Cephalaria syriaca, Phalaris minor, Pisum sativum, Triticum aestivum, Avena fatua* and *Helianthus annuus*

This experiment was conducted to examine the effects of sunflower growth stage on the bioherbicidal activity of aqueous sunflower (shoot and root) extracts on seed germination and early growth, the yield of sunflower shoot and root extract at different growth stages, and to carry out phytochemical analysis of extracts at different sunflower growth stages.

3.1.1 Effects of aqueous shoot extracts from the different growth stages of sunflower plants

3.1.1.1 Effects on seed germination

Analysis of variance showed that germination percentage of all species except *H. annuus* was significantly decreased by aqueous shoot extract from sunflower plants that were two months old. Other treatments were less effective (Figure 24). Germination percentage of *S. cereale, B. napus* and *P. minor* was significantly affected by all sunflower shoot extract treatments (effects on *S. cereale* and *C. syriaca* are illustrated in Figures 29 and 30, respectively). The germination percentages of *A. fatua, T. aestivum, S. cereale* and *B. napus* were significantly reduced by aqueous shoot extract of one month old plants. *T. aestivum* was significantly affected by application of one month, two month and mature stage shoot extracts. Germination of *P. sativum* was significantly reduced by one week, two month and mature stage aqueous shoot extract from sunflower plants. However, there was no significant effect of any of the extracts on germination percentage of *H. annuus*.

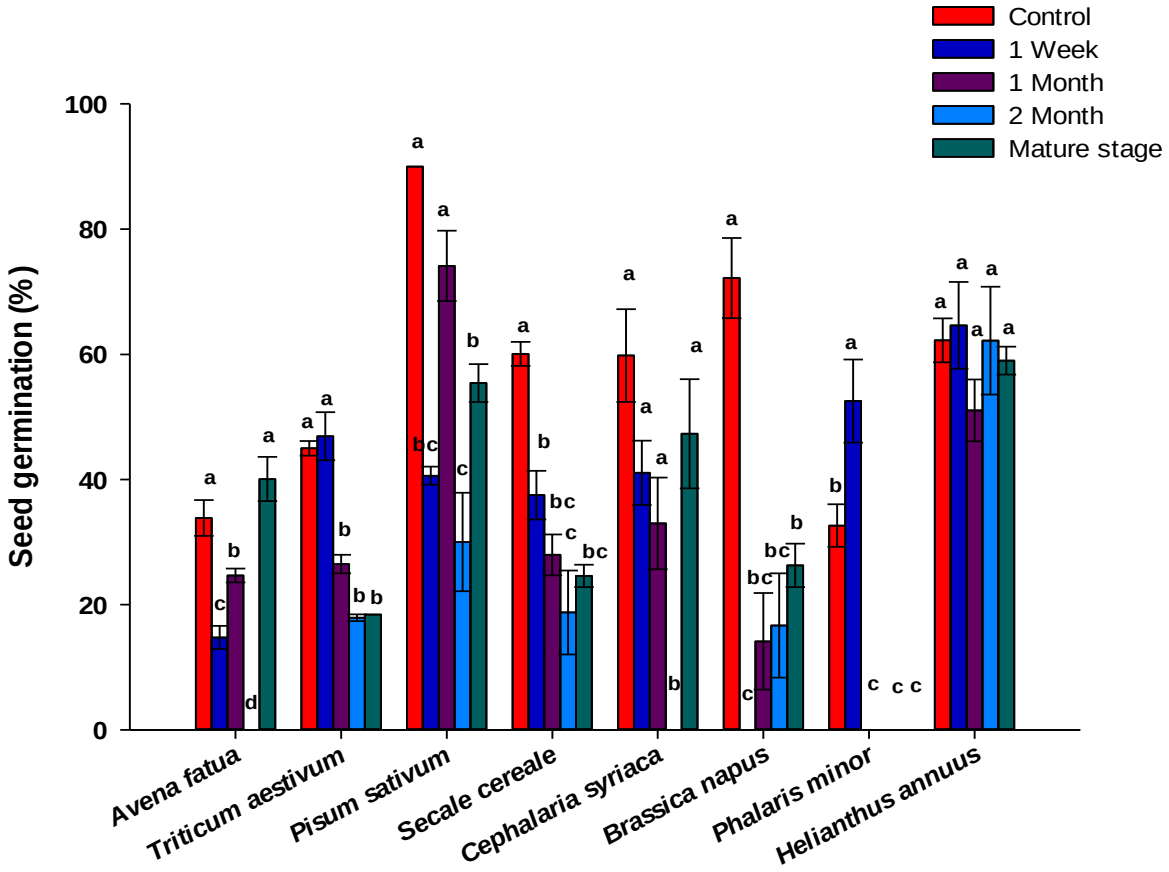


Figure 24 Effect of sunflower shoot extracts (different growth stages) on seed germination.

Extracts of one week, one month, two month and mature stage are made from 10 g of sunflower ground shoot in 100 ml of distilled water. Control: distilled water only. The results are means of triplicate samples. Error bars represent standard errors. Significant differences between means were calculated based on Tukey's test ($P \leq 0.05$). For each species, bars with the same letter are not significantly different. Data analysis was done for each species individually.

3.1.1.2 Effect on shoot length

Figure 25 shows that sunflower aqueous extracts significantly reduced the shoot lengths of most of the plant species. Shoot extracts from two month old sunflowers were most effective. Shoot lengths of *A. fatua*, *C. syriaca* and *P. minor* were significantly reduced by extracts from all four sunflower growth stages but *B. napus* was not significantly affected by the application of mature stage extract (effects on *S. cereale* and *C. syriaca* are illustrated in Figures 29 and 30). There was no significant difference between the effects of any of the shoot extracts, whatever the age of sunflower plants, in *H. annuus*. Overall, two months shoot aqueous extract had the most significant effect on shoot lengths compared with extracts from other stages of sunflower growth.

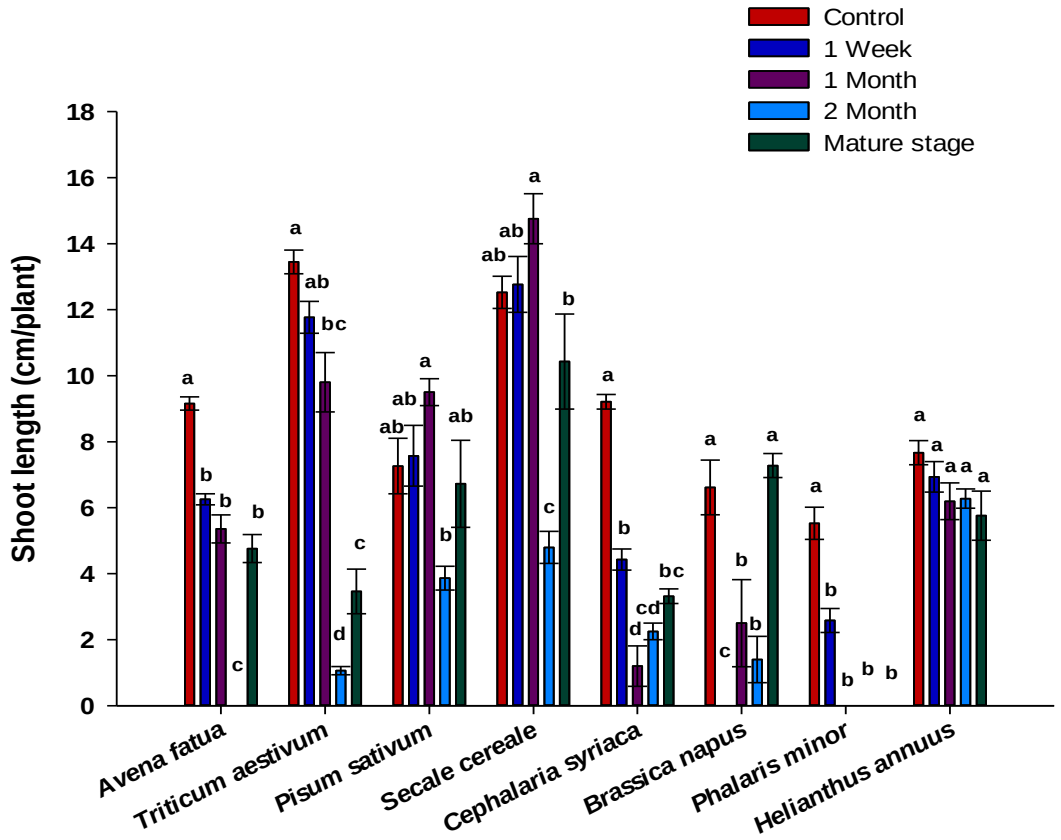


Figure 25 Effect of sunflower shoot extract (different growing stages) on shoot length.

Extracts of one week, one month, two month and mature stage are made from 10 g of sunflower ground shoot in 100 ml of distilled water. Control: distilled water only. The results are means of triplicate samples. Error bars represent standard errors. Significant differences between means were calculated based on Tukey's test ($P \leq 0.05$). For each species, bars with the same letter are not significantly different. Data analysis was done for each species individually.

3.1.1.3 Effect on root length

Sunflower aqueous shoot extracts from all different sunflower plant growth stages significantly reduced root lengths of *A. fatua*, *P. minor* and *C. syriaca*. Overall, extract from two month old sunflower plants had a greater effect than other extracts (one week, one month and mature stage) and significantly reduced root length in most plant species. Overall, the one week aqueous shoot extract of sunflower had the least significant effect on root length (Figure 26).

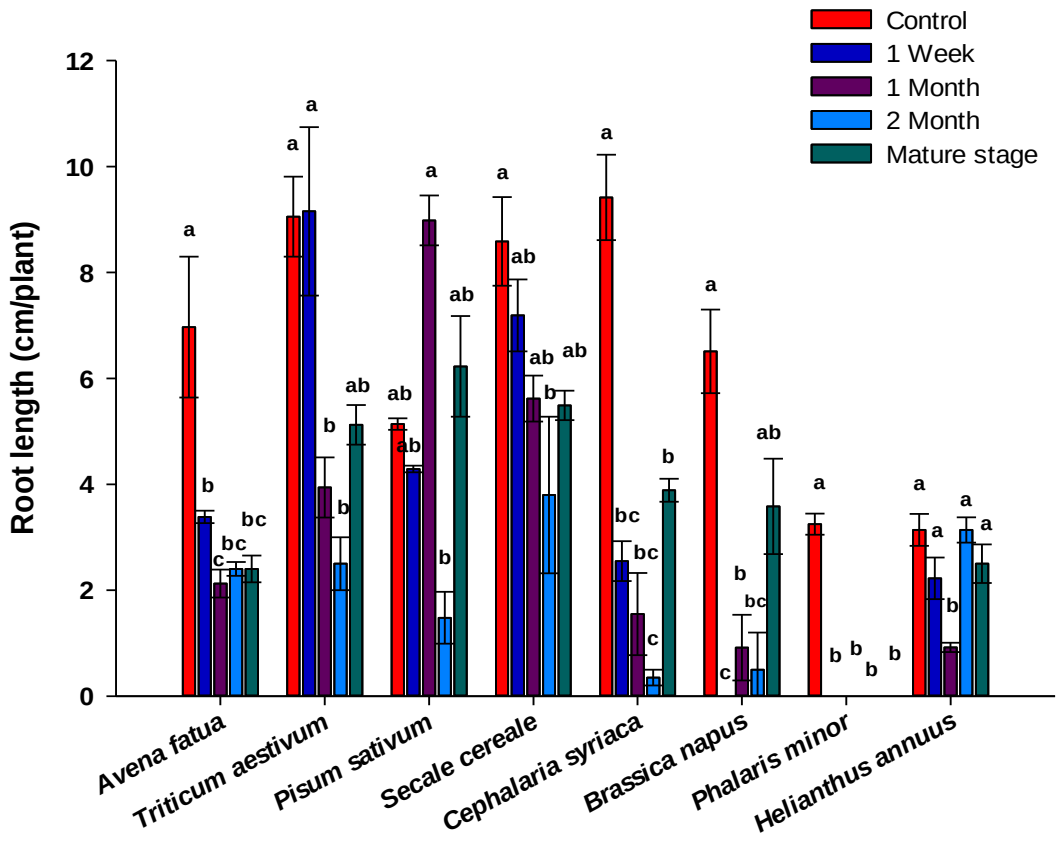


Figure 26 Effect of sunflower shoot extract (different growing stages) on root length.

Extracts of one week, one month, two month and mature stage are made from 10 g of sunflower ground shoot in 100 ml of distilled water. Control: distilled water only. The results are means of triplicate samples. Error bars represent standard errors. Significant differences between means were calculated based on Tukey‘s test ($P \leq 0.05$). For each species, bars with the same letter are not significantly different. Data analysis was done for each species individually.

3.1.1.4 Effect on shoot dry weight

Dry weights of *A. fatua* were significantly affected by aqueous shoot extracts from two month old sunflowers. Furthermore, one month sunflower aqueous shoot extract significantly increased dry weight of *P. sativum* and *S. cereale* (Figure 27). One week sunflower shoot extract reduced dry weight of *B. napus.* Also dry weights of *C. syriaca* were significantly reduced by sunflower aqueous shoot extracts of one month and two months. One month, two month and mature stage aqueous shoot extract of sunflower plants significantly reduced dry weight of *P. minor*. No significant differences were observed between the control and all of the treatments (one week, one month, two month and mature stage) for *H. annuus* and *T. aestivum*.

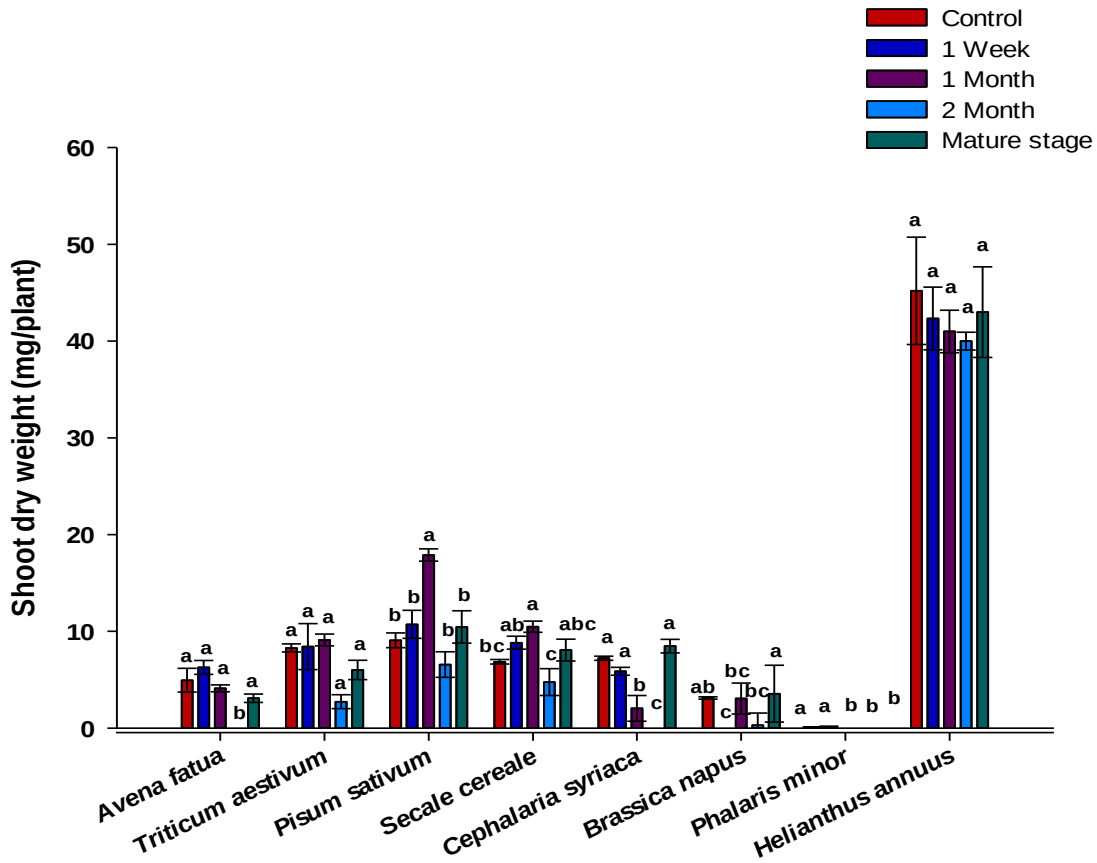


Figure 27 Effect of sunflower shoot extract (different growing stages) on shoot dry weight.

Extracts of one week, one month, two month and mature stage are made from 10 g of sunflower ground shoot in 100 ml of distilled water. Control: distilled water only. The results are means of triplicate samples. Error bars represent standard errors. Significant differences between means were calculated based on Tukey‘s test ($P \leq 0.05$). For each species, bars with the same letter are not significantly different. Data analysis was done for each species individually.

3.1.1.5 Effect on root dry weight

Aqueous extracts from two month old sunflower shoots exhibited significant inhibitory effects on root dry weight of *A. fatua, T. aestivum, C. syriaca* and *P. minor*. One week and one month aqueous shoot extracts significantly reduced root dry weight of *A. fatua, C. syriaca, B. napus,* and *P. minor.* However, there were no significant differences between treatments and control for *P. sativum, S. cereale* and *H. annuus* (Figure 28).

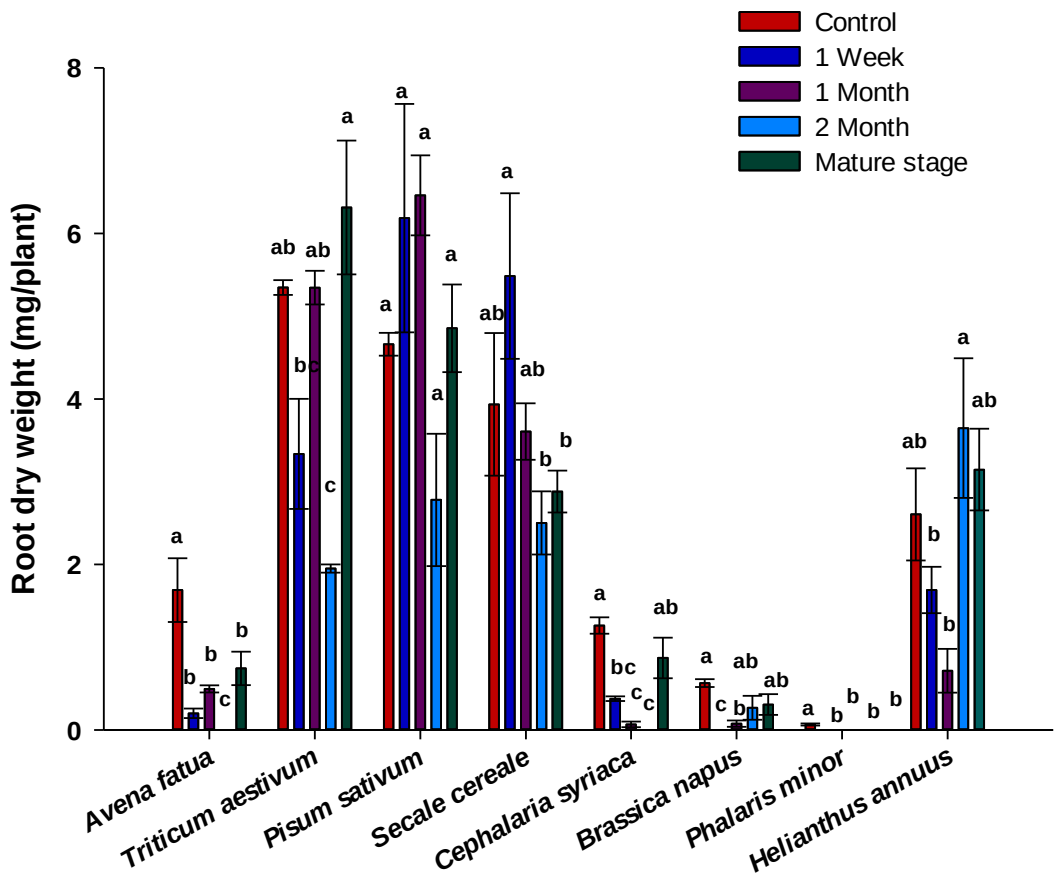


Figure 28 Effect of sunflower shoot extract (different growth stages) on root dry weight.

Extracts of one week, one month, two month and mature stage are made from 10 g of sunflower ground shoot in 100 ml of distilled water. Control: distilled water only. The results are means of triplicate samples. Error bars represent standard errors. Significant differences between means were calculated based on Tukey‘s test ($P \leq 0.05$). For each species, bars with the same letter are not significantly different. Data analysis was done for each species individually.

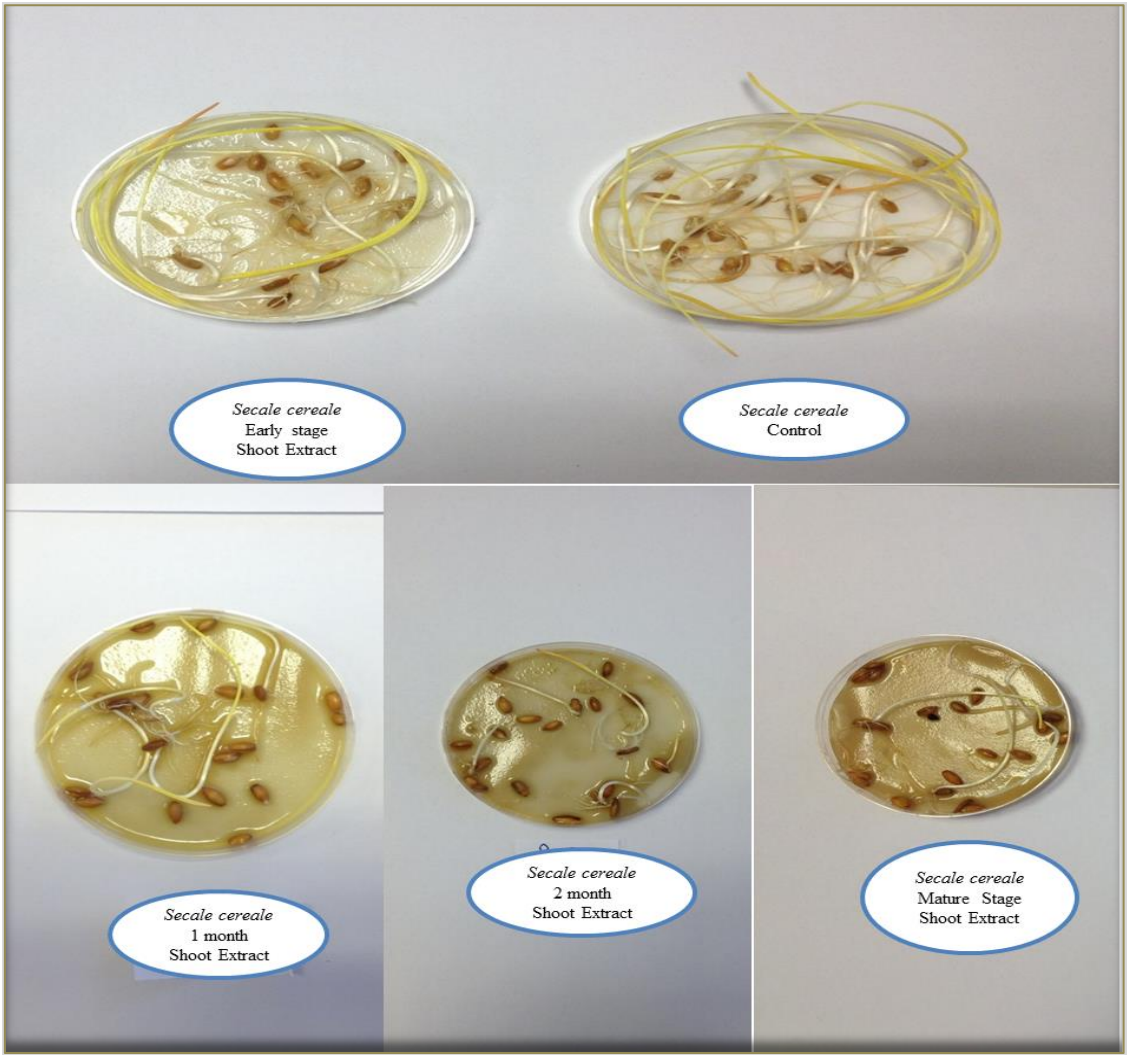


Figure 29 Effects of sunflower shoot extract (different growth stages) on germination and early growth of *Secale cereale*.

Figure 30 Effects of sunflower extract (different growth stages) on germination and early growth of *Cephalaria syriaca.*

3.1.2 Effects of aqueous root extracts from the different growth stages of sunflower plants on germination and early growth of *Brassica napus, Secale cereale, Cephalaria syriaca, Phalaris minor, Pisum sativum, Triticum aestivum, Avena fatua* and *Helianthus annuus*

3.1.2.1 Effect on seed germination

One week aqueous root extract significantly reduced germination percentage of *S. cereale*, *C. syriaca* and *B. napus* (Figure 31). One month aqueous root extract significantly inhibited germination percentage of *Secale cereale* (illustrated in Figure 36), *B. napus, P. minor* and *H. annuus*. Additionally, two month aqueous root extract of sunflower reduced seed germination of *S. cereale, P. minor,* and *H. annuus*. Also *S. cereale* and *P. minor* were significantly affected by mature root aqueous extract. However, there was no significant reduction in seed germination of *A. fatua, T. aestivum* and *P. sativum* plants.

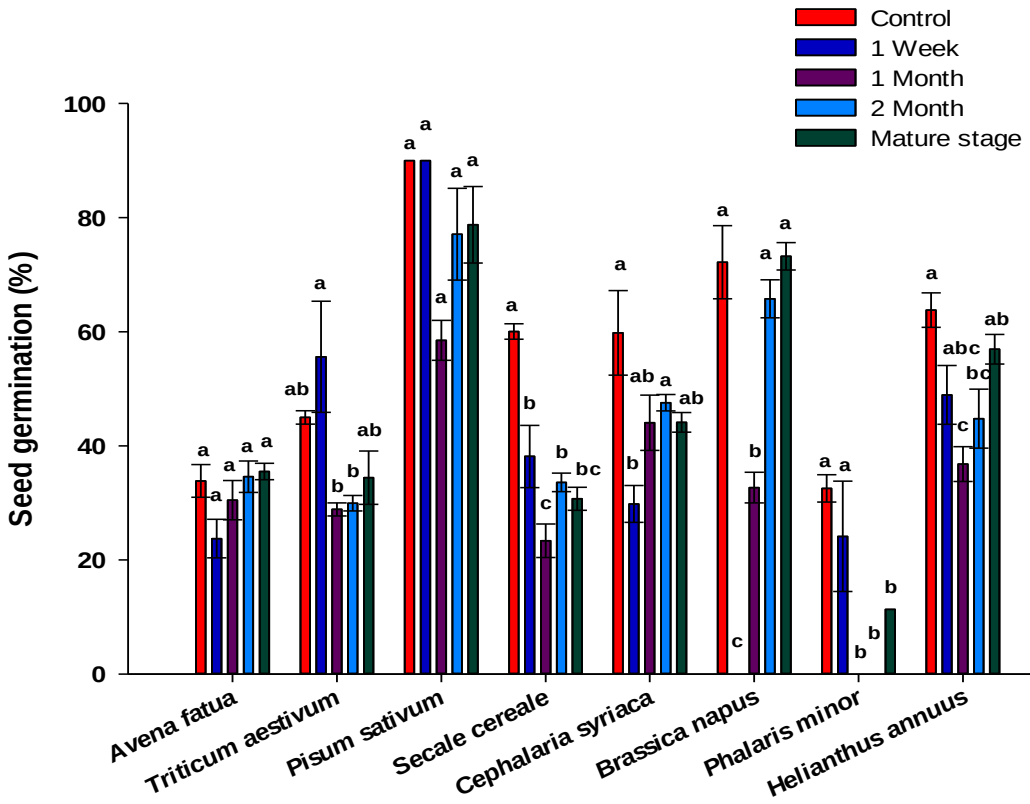


Figure 31 Effect of sunflower root extract (different growth stages) on seed germination. Extracts of one week, one month, two month and mature stage are made from 10 g of sunflower ground root in 100 ml of distilled water. Control: distilled water only. The results are means of triplicate samples. Error bars represent standard errors. Significant differences between means were calculated based on Tukey‘s test ($P \leq 0.05$). For each species, bars with the same letter are not significantly different. Data analysis was done for each species individually.

3.1.2.2 Effect on shoot length

Aqueous root extract from one month old sunflowers significantly reduced shoot length of *P. sativum, S. cereale* and *P. minor* (illustrated in Figure 36) and one week extract significantly reduced shoot length of *C. syriaca* (illustrated in Figure 37). Similarly, *B. napus* was significantly affected by one week and one month root extracts (Figure 32). Also, two month old sunflower aqueous extract significantly decreased shoot length of *P. minor*. However, that of *B. napus* was significantly increased, and mature root extract significantly increased shoot length of *P. sativum, S. cereale,* and *B. napus*. None of the extracts significantly affected shoot lengths of *A. fatua, T. aestivum* and *H. annuus* plants.

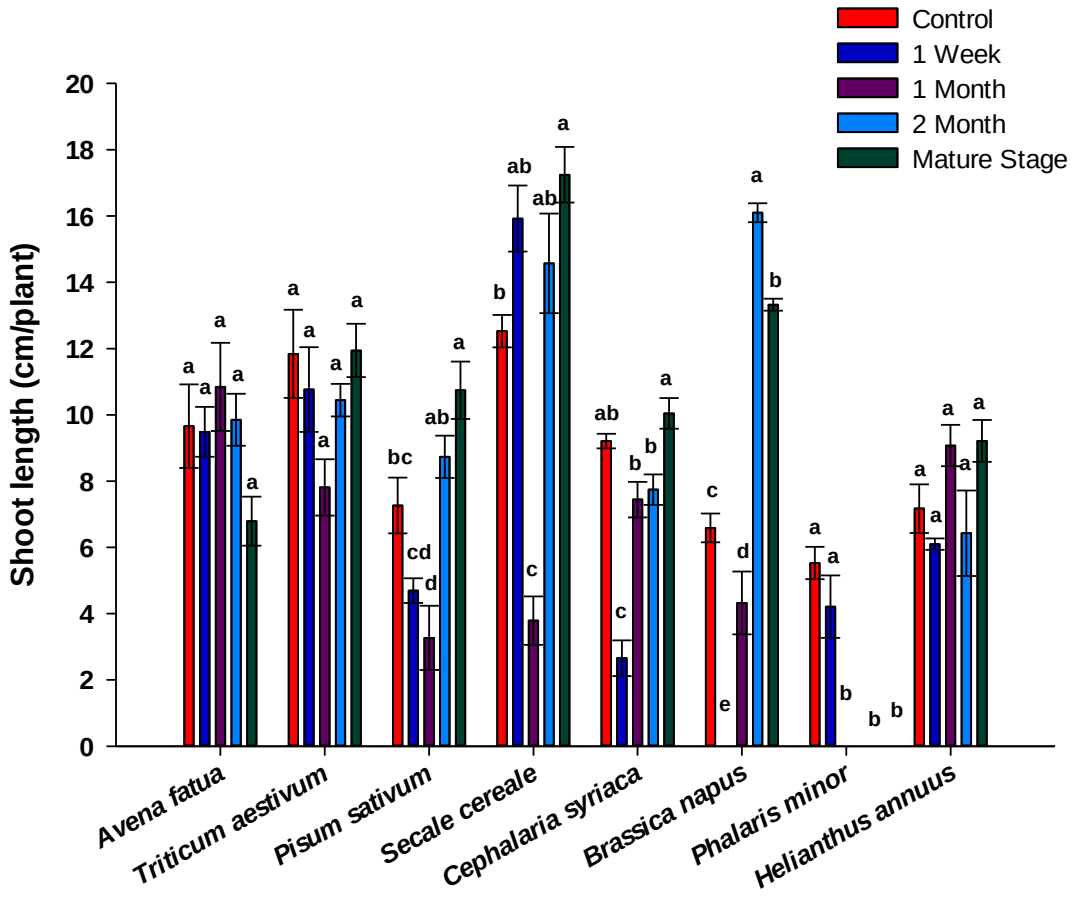


Figure 32 Effect of sunflower root extract (different growth stages) on shoot length.

Extracts of one week, one month, two month and mature stage are made from 10 g of sunflower ground root in 100 ml of distilled water. Control: distilled water only. The results are means of triplicate samples. Error bars represent standard errors. Significant differences between means were calculated based on Tukey‘s test ($P \leq 0.05$). For each species, bars with the same letter are not significantly different. Data analysis was done for each species individually.

3.1.2.3 Effect on root length

One week sunflower aqueous root extract significantly reduced root length of *C. syriaca*, *B. napus* and *P. minor* (illustrated in Figure 37), whereas one month aqueous root extract significantly reduced root length of *T. aestivum*, *S. cereale*, *B. napus* and *P. minor* (Figure 33). Furthermore, two month and mature stage root aqueous extracts significantly reduced root length of *P. minor*. However, root lengths of *P. sativum* and *B. napus* were significantly increased by mature stage root extract. There was no significant difference between control and root water extracts for *A. fatua* and *H. annuus*.

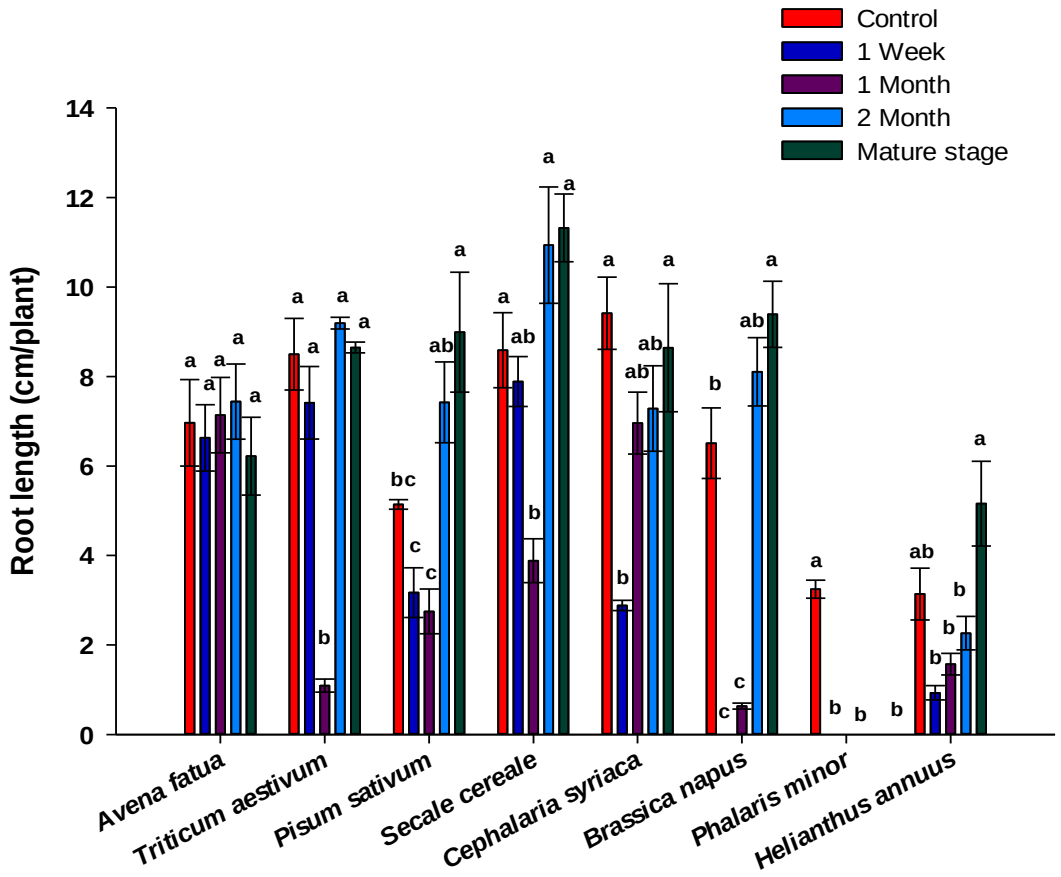


Figure 33 Effect of sunflower root extract (different growth stages) on root length.

Extracts of one week, one month, two month and mature stage are made from 10 g of sunflower ground root in 100 ml of distilled water. Control: distilled water only. The results are means of triplicate samples. Error bars represent standard errors. Significant differences between means were calculated based on Tukey‘s test ($P \leq 0.05$). For each species, bars with the same letter are not significantly different. Data analysis was done for each species individually.

3.1.2.4 Effect on shoot dry weight

Figure 34 shows that one week and one month aqueous root extracts significantly reduced shoot dry weight of *B. napus* whereas two month and mature stage root extracts significantly increased shoot dry weight. However, shoot dry weights of all other species treated with aqueous root extracts were not significantly different from the controls.

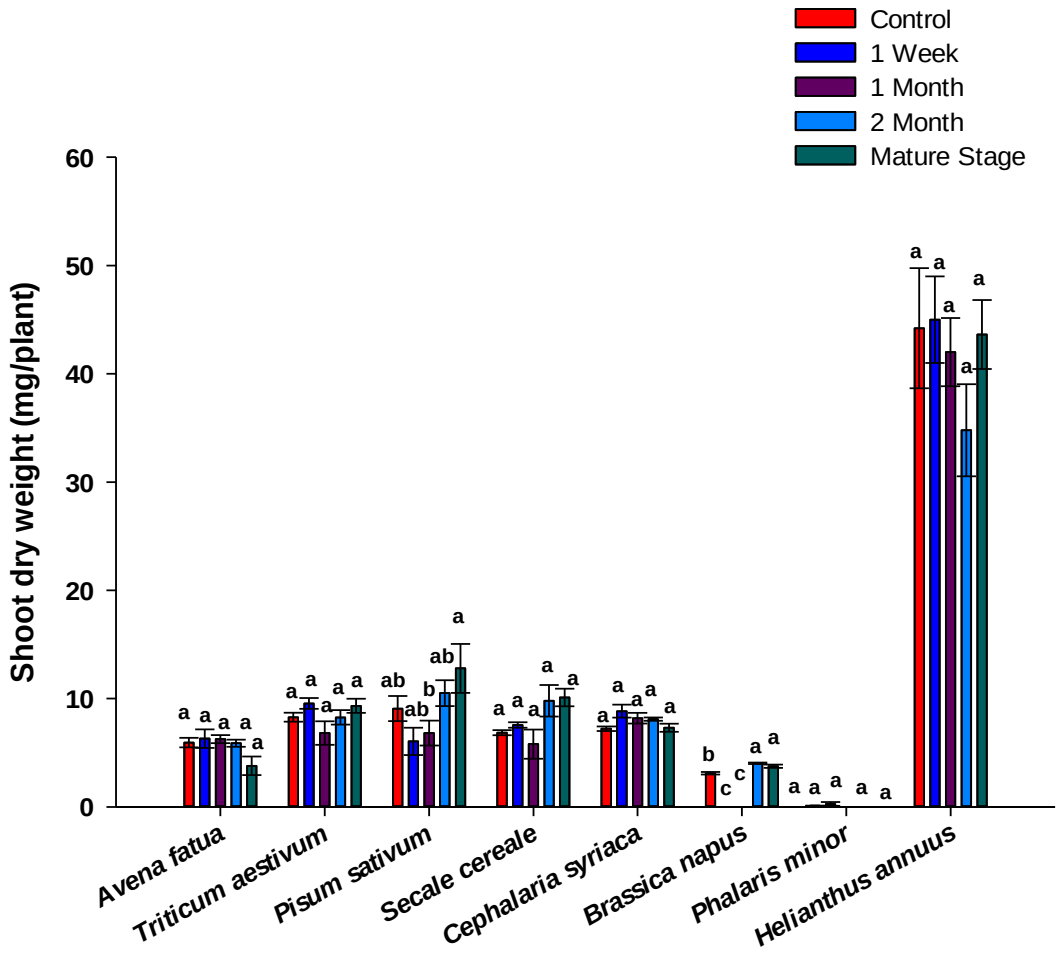


Figure 34 Effect of sunflower root extracts (different growth stages) on shoot dry weight.

Extracts of one week, one month, two month and mature stage are made from 10 g of sunflower ground root in 100 ml of distilled water. Control: distilled water only. The results are means of triplicate samples. Error bars represent standard errors. Significant differences between means were calculated based on Tukey's test ($P \leq 0.05$). For each species, bars with the same letter are not significantly different. Data analysis was done for each species individually.

3.1.2.5 Effect on root dry weight

Aqueous extract of one week old sunflower roots significantly reduced root dry weight of *A. fatua*, *T. aestivum*, *C. syriaca*, *B. napus* and *P. minor* (Figure 35). Root dry weights of *A. fatua*, *P sativum*, *S. cereale*, *B. napus*, and *P. minor* were significantly affected by one month aqueous root extract. Root dry weight of *P. minor* was also significantly reduced by two month and mature stage root extract treatments. There were no significant differences between the control treatment and any root extract treatments for *H. annuus*.

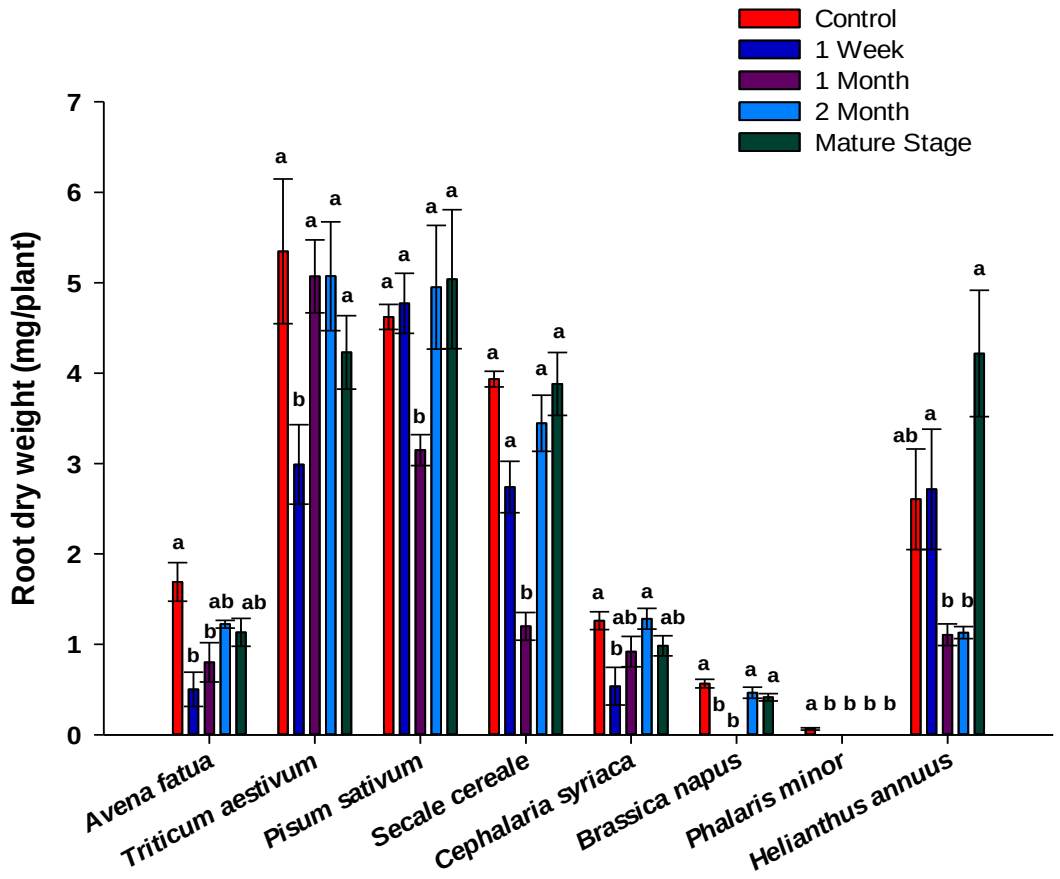


Figure 35 Effect of sunflower root extracts (different growth stages) on root oven dry weight. Extracts of one week, one month, two month and mature stage are made from 10 g of sunflower ground root in 100 ml of distilled water. Control: distilled water only. The results are means of triplicate samples. Error bars represent standard errors. Significant differences between means were calculated based on Tukey‘s test ($P \leq 0.05$). For each species, bars with the same letter are not significantly different. Data analysis was done for each species individually.

Figure 36 Effects of sunflower root extracts (different growth stages) on seed germination and early growth of *Secale cereale*.

Figure 37 Effect of sunflower root extracts (different growth stages) on seed germination and early growth of *Cephalaria syriaca*.

3.1.3 Freeze-dried sunflower shoot and root extracts at different growth stages

The yields of sunflower shoot and root extracts at different growth stages (one week, one month, two month and mature) were studied (Table 2). Two month shoot and one week root extract gave more yield than other samples (18.9%, 14.22% respectively). The lowest shoot freeze-dried weight was for one month extract. Moreover, the lowest root freeze-dried weight was for mature stage root extract (4.31%).

Table 2 Freeze-dried sunflower shoot and root extracts at different growth stages.

	Shoot Extract Freeze-dried (g)	Yield (%)	Root Extract Freeze-dried (g)	Yield (%)
1 Week	1.197	11.97	1.42	14.22
1 Month	1.128	11.28	0.814	8.14
2 Month	1.89	18.9	0.742	7.42
Mature stage	1.542	15.42	0.431	4.31

3.1.4 Screening of phytochemicals in sunflower shoot and root extracts at different growth stages

Qualitative analysis of phytochemicals from sunflower shoot and root extracts at one week, one month, two month and mature stages showed that tannins, terpenoids, saponins, phenolics and flavonoids were present in both shoot and root extracts at most growth stages, while phlobatannins were only present in root extracts at different growth stages except one week. However, alkaloids were not detected in sunflower shoot and root extracts (Table 3).

Table 3 Qualitative chemical tests of sunflower shoot and root extracts at different growth stages.

	Tannins	Phlobatannins	Terpenoids	Alkaloids	Saponins	Phenolics	Flavonoids
Shoot Extract							
1 Week	+	-	+	-	+	++	+
1 Month	+	-	+	-	+	++	++
2 Month	+	-	+	-	+	+++	++
Mature stage	+	-	+	-	+	++	++
Root Extract							
1 Week	-	-	+	-	+	+	+
1 Month	+	+	+	-	+	+	+
2 Month	+	+	+	-	+	+	+
Mature stage	+	+	+	-	+	+	+

Experiment 2

3.2.1 Effects of different concentrations of sunflower shoot extract made from plants at the mature stage on germination and early growth of rapeseed (*Brassica napus*), rye (*Secale cereale*), Syrian Cephalaria (*Cephalaria syriaca*) and littleseed canary grass (*Phalaris minor*)

This experiment investigated the effects of different concentrations of aqueous shoot extracts at the mature stage on seed germination and seedling growth of two monocot and two dicot plant species and the effects of sunflower shoot extract on mitotic index and cell elongation.

3.2.1.1 Effect on seed germination

Statistical analysis using Tukey's test shows that 10% and 5% concentrations of sunflower aqueous shoot extract significantly ($P < 0.001$) suppressed seed germination of *B. napus* (illustrated in Figure 43), *C. syriaca* and *P. minor* as compared with the controls (Figure 38). Sunflower aqueous shoot extract (mature stage) did not significantly reduce seed germination of *S. cereale*.

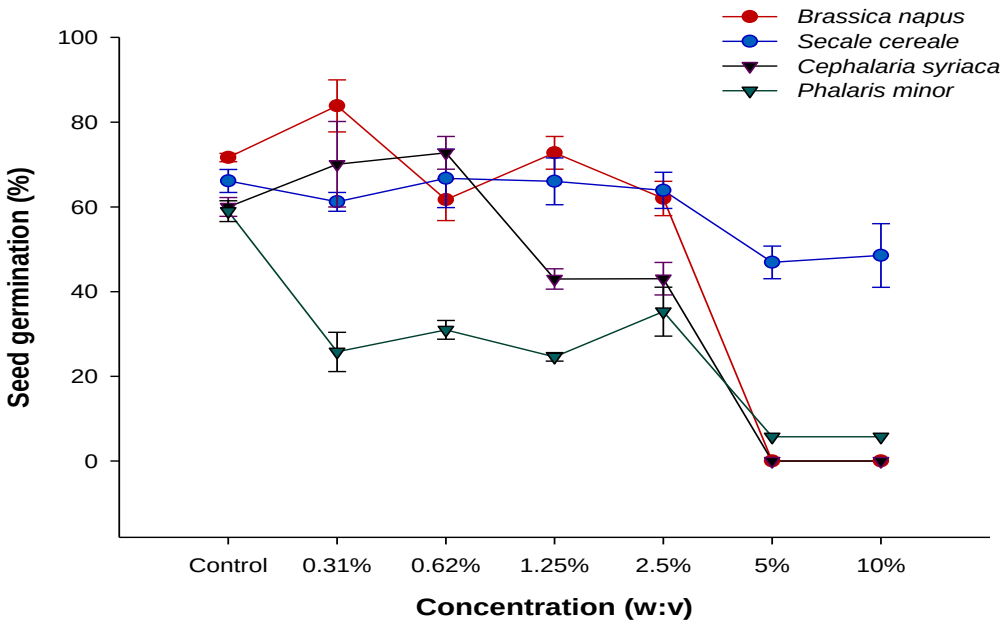


Figure 38 Effect of sunflower shoot extract (mature growth stage) on seed germination.

Shoot extract was diluted with distilled water. Control: distilled water. The results are means of triplicate samples. Error bars represent standard errors. Data analysis was done for all species collectively.

3.2.1.2 Effect on shoot length

Shoot growth of *B. napus* (illustrated in Figure 43) was completely inhibited ($P < 0.001$) by 5% and 10% concentrations of sunflower aqueous shoot extract (Figure 39). Minimum inhibition was with the concentration of 0.62%. Shoot length of *C. syriaca* was significantly reduced by concentrations of 1.25%, 2.50%, 5%, and 10%. Shoot lengths of *P. minor* and *S. secale* were only significantly reduced by the highest concentration (10%).

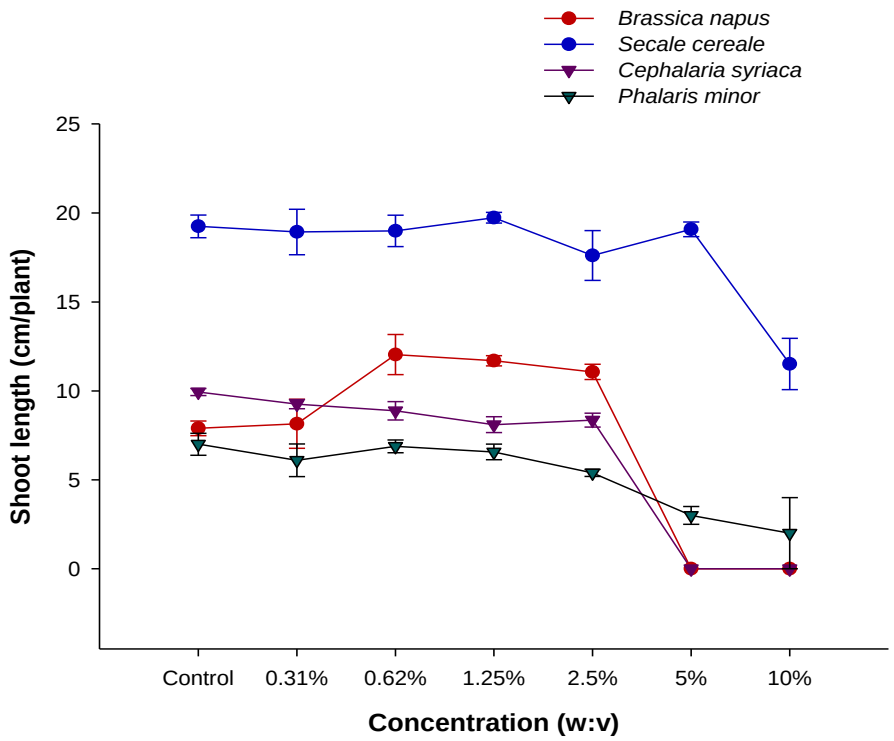


Figure 39 Effect of sunflower shoot extract (mature growth stage) on shoot length.

Shoot extract was diluted with distilled water. Control: distilled water. The results are means of triplicate samples. Error bars represent standard errors. Data analysis was done for all species collectively.

3.2.1.3 Effect on root length

Figure 40 shows that 5% and 10% aqueous shoot extract prevented root growth of *B. napus* and *C. syriaca*. Extract at 10% significantly ($P < 0.001$) reduced root length of *S. cereale* and *P. minor*; 5% extract also significantly reduced root length of *P. minor* but to a lesser extent.

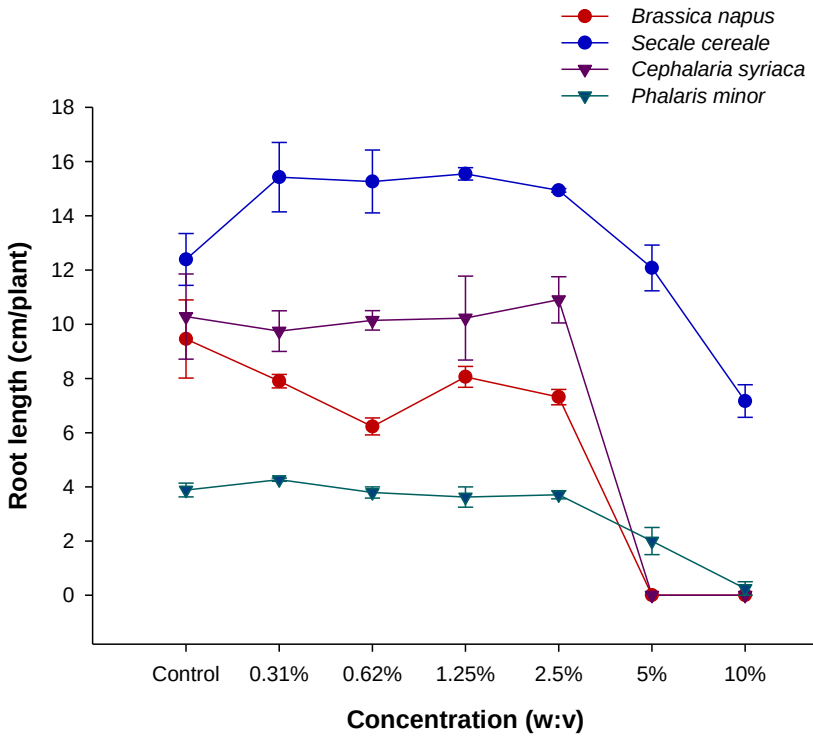


Figure 40 Effect of sunflower shoot extract (mature growth stage) on root length.

Shoot extract was diluted with distilled water. Control: distilled water. The results are means of triplicate samples. Error bars represent standard errors. Data analysis was done for all species collectively.

3.2.1.4 Effect on shoot dry weight

Oven dry weights of *B. napus* and *C. syriaca* shoots were significantly ($P < 0.001$) decreased by the 5% and 10% concentrations of aqueous shoot extract of mature stage. 1.25% and 10% aqueous shoot extract significantly reduced shoot oven dry weight of *P. minor*. There were no significant differences between the control and all aqueous shoot extract concentrations in *Secale cereale* (Figure 41).

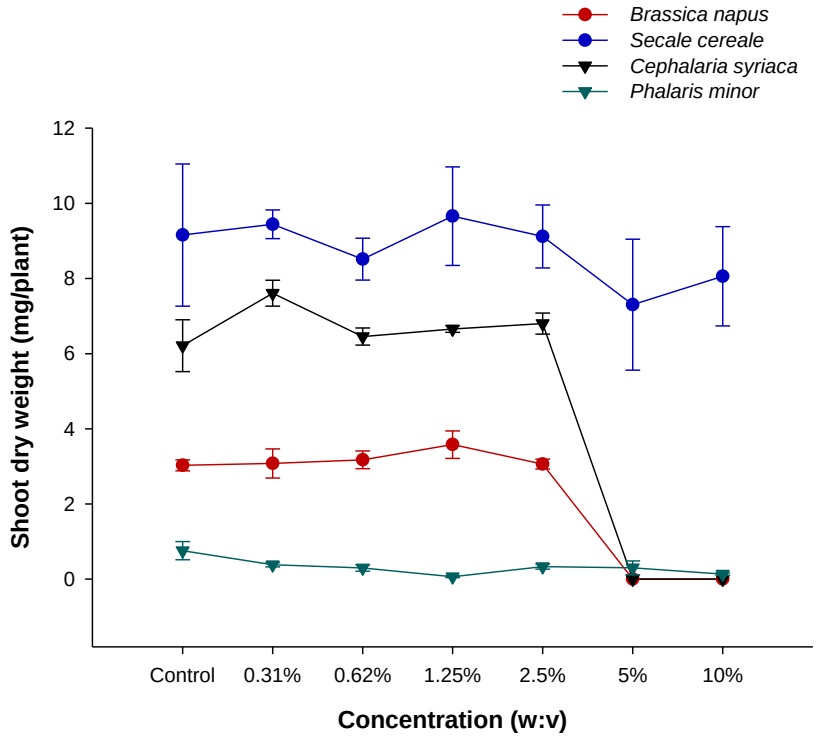


Figure 41 Effect of sunflower shoot extract (mature growth stage) on shoot dry weight.

Shoot extract was diluted with distilled water. Control: distilled water. The results are means of triplicate samples. Error bars represent standard errors. Data analysis was done for all species collectively.

3.2.1.5 Effect on root dry weight

Figure 42 shows the effects of different concentrations of sunflower aqueous shoot extract (mature stage) on root dry weight of *B. napus, S. cereale, C. syriaca* and *P. minor*. Root dry weights of *B. napus, C. syriaca* and *P. minor* were significantly ($P < 0.001$) affected by 2.5%, 5%, and 10% concentrations of aqueous shoot extract of sunflower (mature stage). Also *P. minor* root growth was completely inhibited at the different concentrations of sunflower shoot extract compared with the control. There were no significant differences between treatments and the control for *S. cereale.*

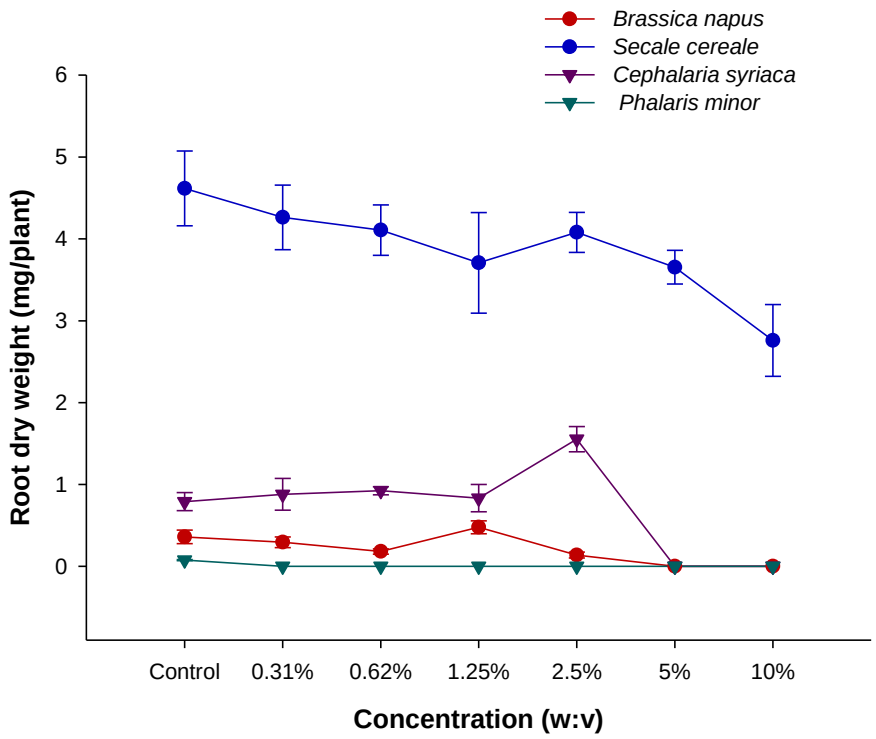


Figure 42 Effect of sunflower shoot extract (mature growth stage) on root dry weight.

Shoot extract was diluted with distilled water. Control: distilled water. The results are means of triplicate samples. Error bars represent standard errors. Data analysis was done for all species collectively.

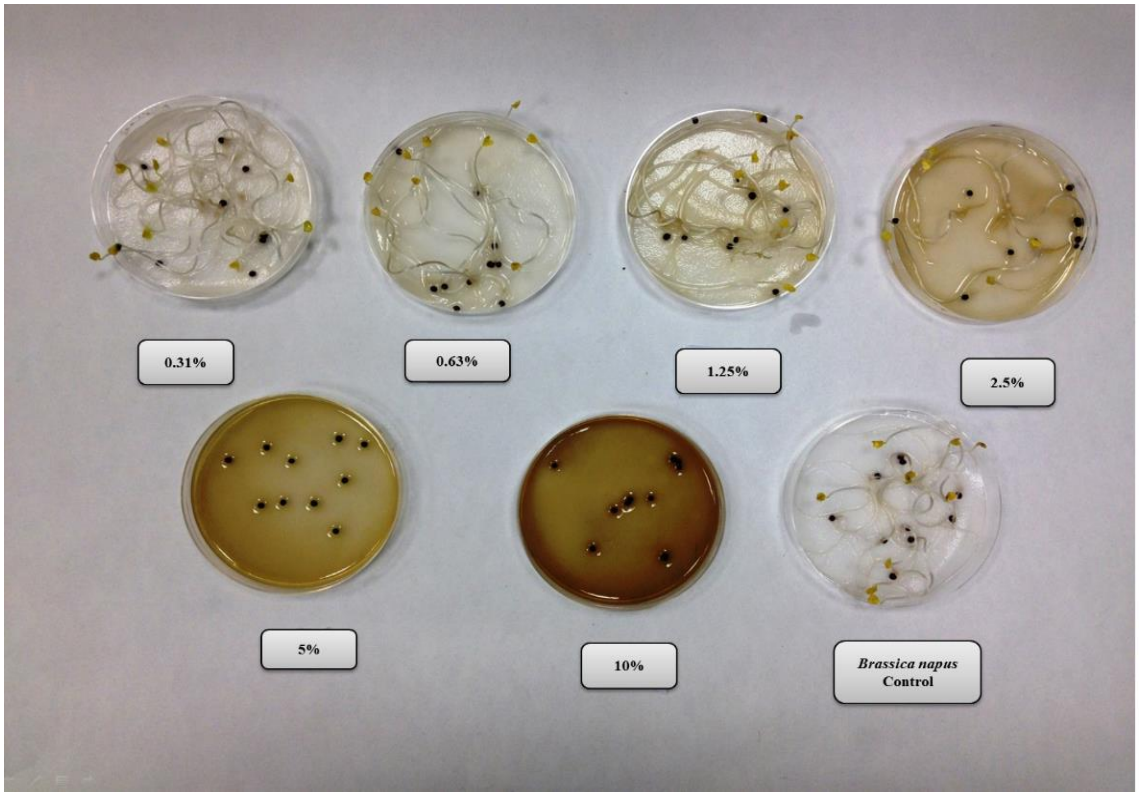


Figure 43 Effect of sunflower shoot extract (mature growth stage) on seed germination and early growth of *Brassica napus.*

Shoot extract was diluted with distilled water. Control: distilled water. Shoot extract concentrations: 0.31, 0.63, 1.25, 2.5, 5, and 10%. The experiment was conducted in growth chamber. The germination and growth test lasted seven days.

3.2.2 Total phenolic and alkaloid detection from aqueous shoot extract (mature stage) of sunflower by using spectrophotometer

Total phenolic concentration (gallic acid equivalent) of 500 mg freeze-dried sunflower shoot extract (mature stage) was determined by using the Folin-Ciocalteu method. The concentration of total phenolic compounds in sunflower shoot extract was 424.8 µg/ml. However, testing for alkaloids gave no evidence that sunflower shoot extract contained alkaloids.

3.2.3 Effect of sunflower aqueous shoot extract on mitotic index and cell elongation of *Avena fatua* root meristems

3.2.3.1 Effect of sunflower aqueous shoot extract on mitotic index

In the presence of sunflower aqueous shoot extract (two month old), wild oat (*A. fatua*) root tips showed a reduced mitotic index of 24 compared with 30.33 in control root tips (Figure 44). However, there was no statistically significant difference in mitotic index between control and test treatments. Statistical analysis indicated that there was no significant difference in proportions of cells in the different mitotic phases (prophase, metaphase, anaphase, and telophase) between control and test treatments with applied sunflower aqueous shoot extract (Table 6).

A – Control meristems

$$\text{Mitotic Index} = \frac{(P + M + A + T)}{\text{Total no. of cell scored}} \times 100$$

$$\text{Mitotic Index} = \frac{(24 + 19 + 21 + 27)}{300} \times 100 = 30.33$$

B- Test meristems

$$\text{Mitotic Index} = \frac{(P + M + A + T)}{\text{Total no. of cells scored}} \times 100$$

$$\text{Mitotic Index} = \frac{(25 + 20 + 14 + 13)}{300} \times 100 = 24$$

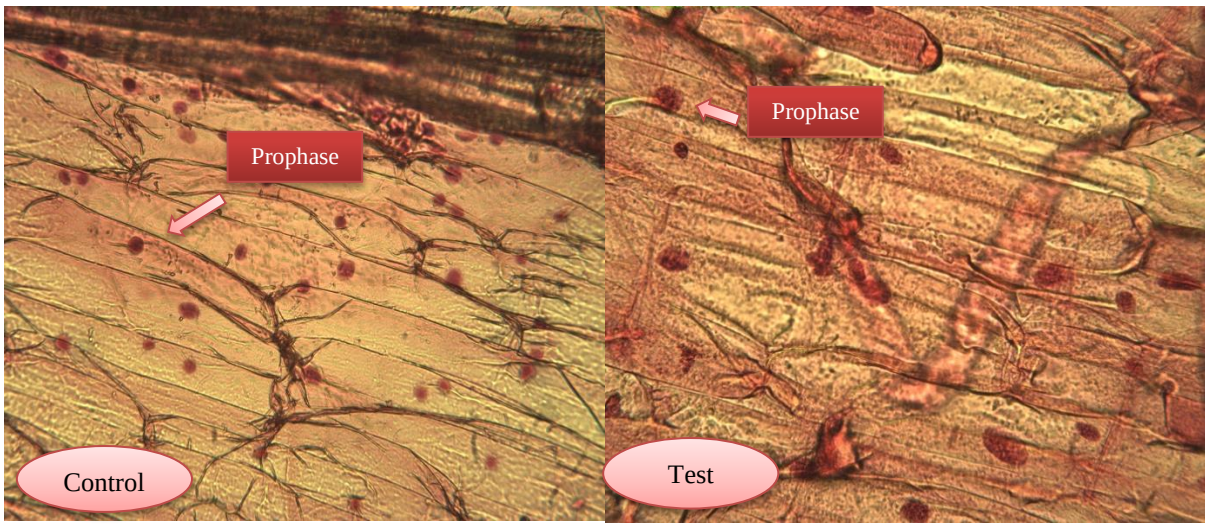


Figure 44 Effect of sunflower two month aqueous shoot extract on mitotic index of *Avena fatua*.

3.2.3.2 Effect of sunflower aqueous shoot extract on cell elongation of *Avena fatua* meristems

Sunflower aqueous shoot extract significantly reduced cell length of the root meristems of *Avena fatua* compared with the control (Figure 45).

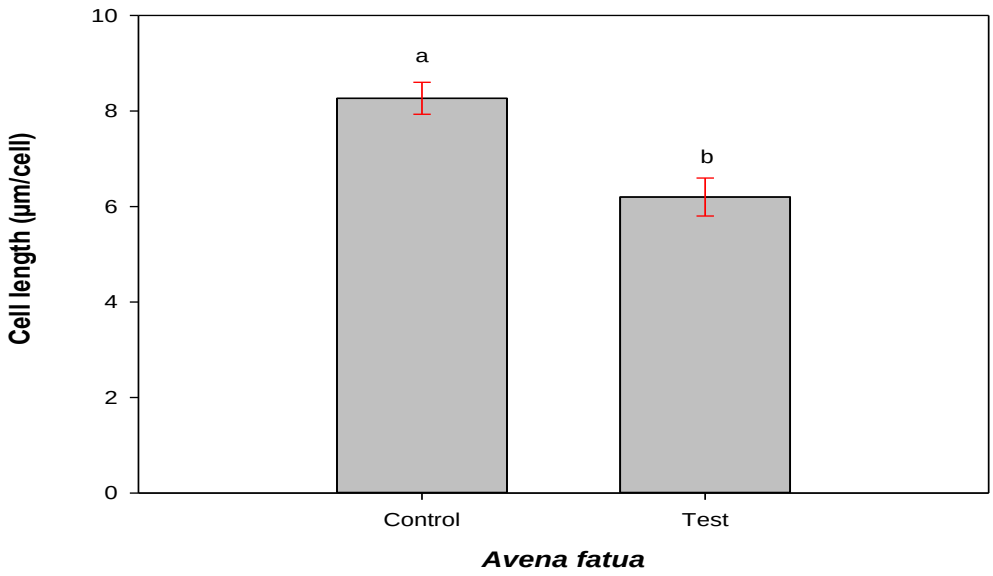


Figure 45 Effect of sunflower two month shoot aqueous extract on cell elongation of *Avena fatua*. Bars show mean ± SE. Bars with same letter are not significantly different.

Experiment 3

The aim of this experiment was to compare the effects of sunflower two month shoot aqueous extract with sunflower ground shoot incorporated into soil on germination and growth of *Brassica napus*, *Avena fatua*, *Cephalaria syriaca*, and *Phalaris minor*. Effects on seed germination and growth, soil physiological properties, and chlorophyll a and chlorophyll b content were measured.

3.3 Effect of two month sunflower shoot aqueous extract and ground shoot on germination and early growth of rapeseed (*Brassica napus*), wild oat (*Avena fatua*), Syrian Cephalaria (*Cephalaria syriaca*), and littleseed canary grass (*Phalaris minor*)

3.3.1 Effects of sunflower ground shoot on germination and early growth of *B. napus*, *A. fatua*, *C. syriaca* and *P. minor*

3.3.1.1 Effects on seed germination

Germination percentage of *C. syriaca* was significantly reduced by sunflower two month ground shoot at concentrations of 6 g and 9 g/pot (illustrated in Figure 49). However, there were no significant differences between the control and other treatments in *B. napus*, *A. fatua* and *P. minor* (Figure 46).

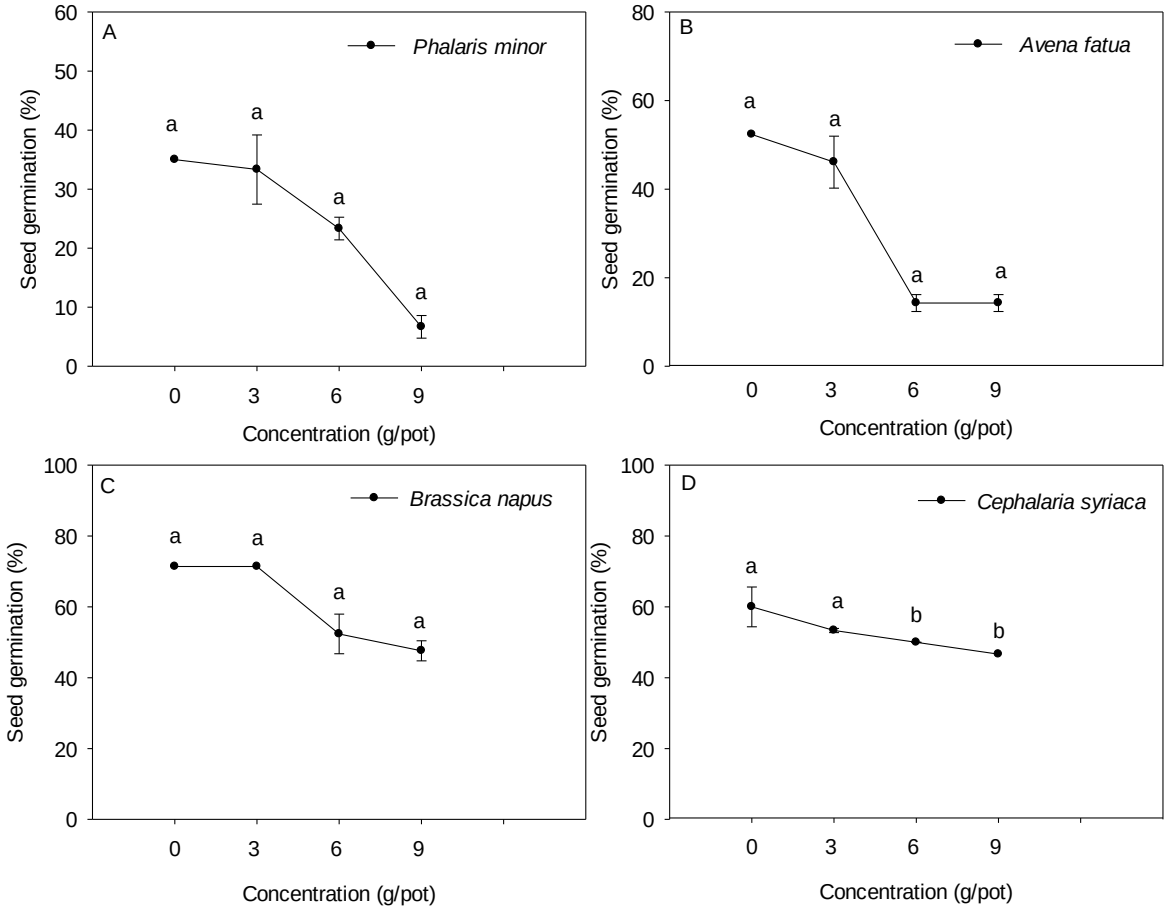


Figure 46 Effects of sunflower ground shoot on seed germination of *Brassica napus*, *Avena fatua*, *Cephalaria syriaca* and *Phalaris minor.*

The results are means of triplicate samples. Error bars represent standard errors. Significant differences between means were calculated based on Tukey's test ($P \leq 0.05$). For each species, points with the same letter are not significantly different. Data analysis was done for all species collectively.

3.3.1.2 Effects on shoot length

Figure 47 shows that sunflower ground shoot significantly reduced the shoot lengths of *A. fatua, C. syriaca* and *B. napus* at higher concentrations (illustrated in Figure 50). Although there was a difference between control and treated plants for *P. minor*, this difference was not significant

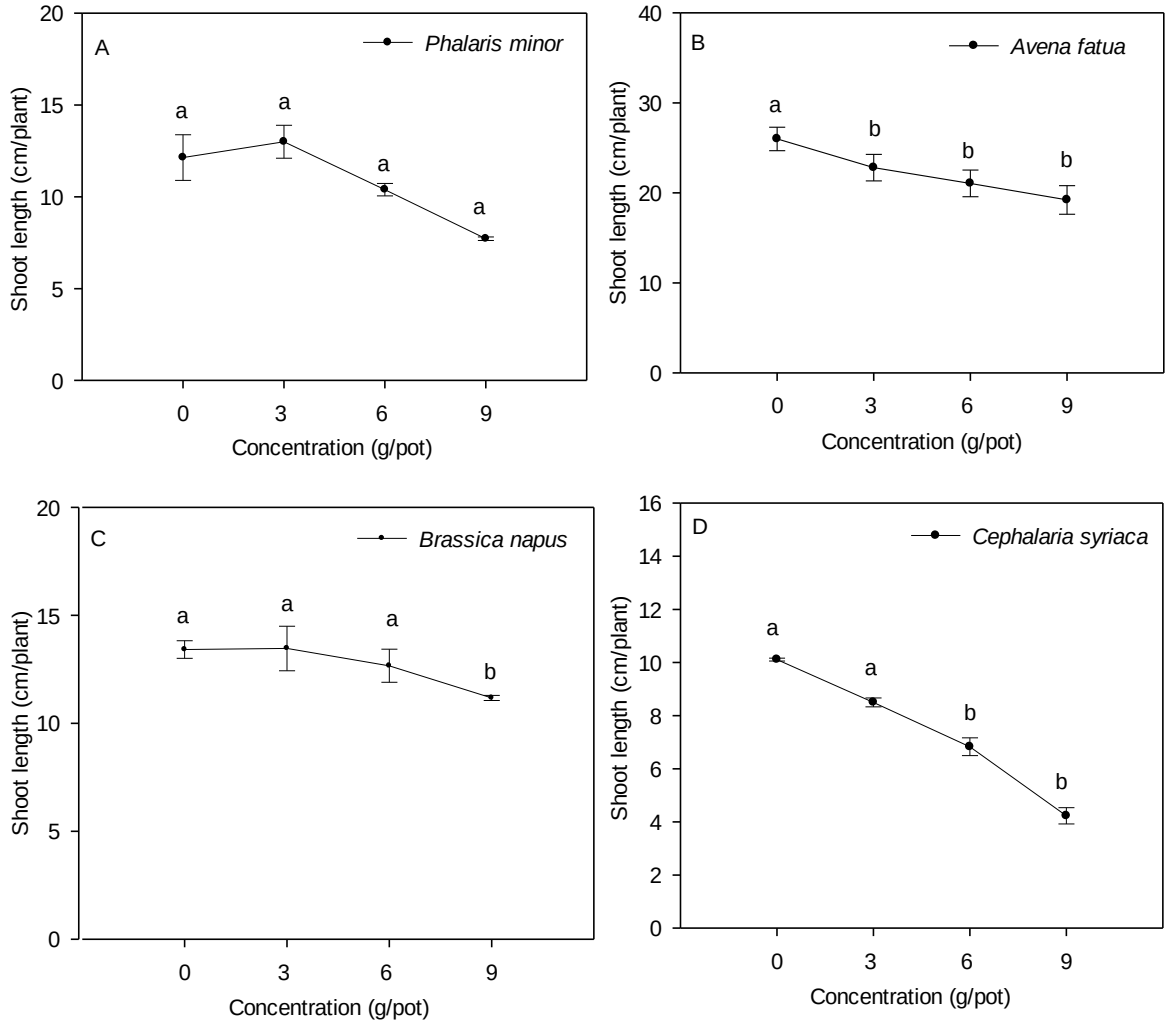


Figure 47 Effect of sunflower ground shoot on shoot length of *Brassica napus, Avena fatua, Cephalaria syriaca* and *Phalaris minor*.

The results are means of triplicate samples. Error bars represent standard errors. Significant differences between means were calculated based on Tukey's test ($P \leq 0.05$). For each species, points with the same letter are not significantly different. Data analysis was done for all species collectively.

3.3.1.3 Effects on root length

Figure 48 shows that two month sunflower ground shoot at 6 and 9 g/pot significantly reduced root length of *A. fatua*, *C. syriaca* (illustrated in Figure 50) and *P. minor*. The highest concentration of sunflower two month ground shoot had a greater effect than other concentrations. Root length of *B. napus* was not significantly affected by sunflower two month residue.

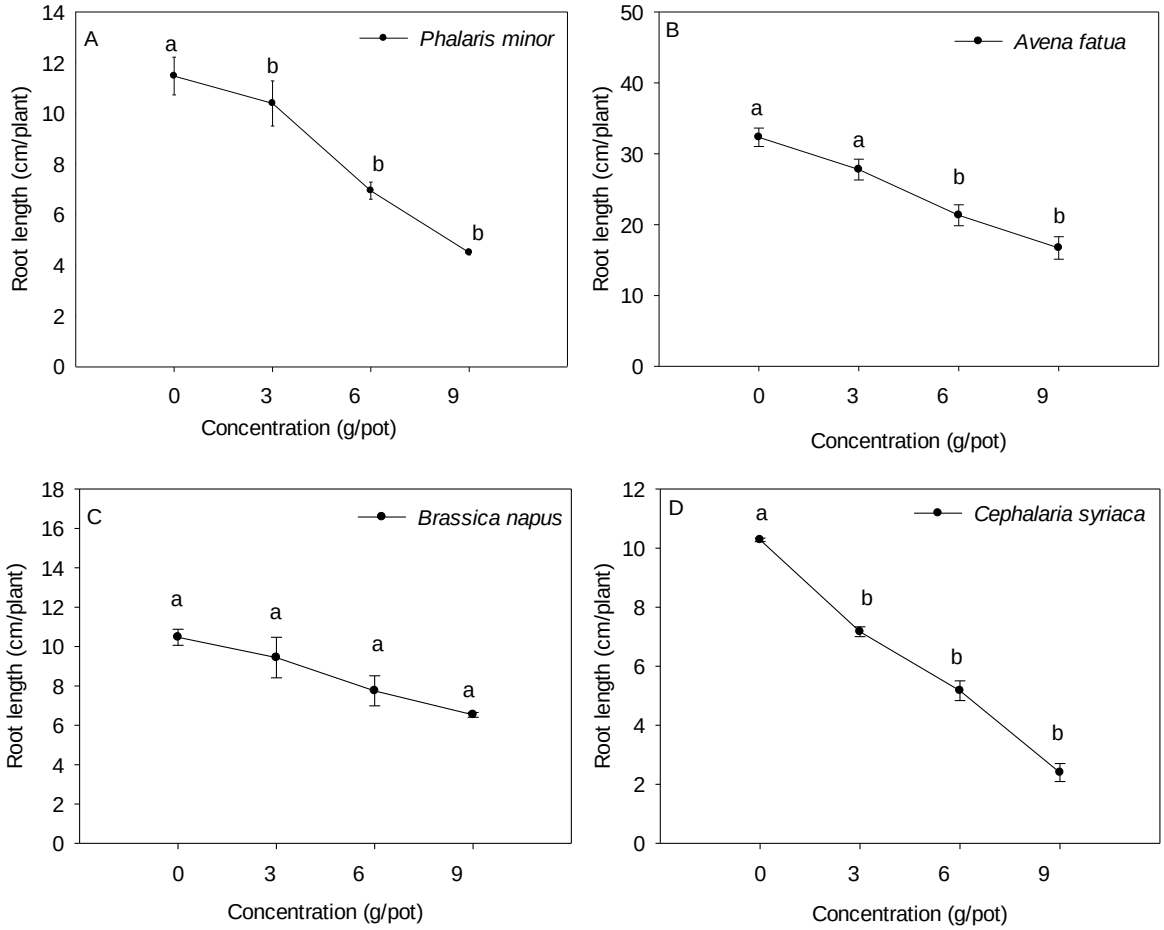


Figure 48 Effect of sunflower ground shoot on root lengths of *Brassica napus, Avena fatua, Cephalaria syriaca* and *Phalaris minor.*

The results are means of triplicate samples. Error bars represent standard errors. Significant differences between means were calculated based on Tukey's test ($P \leq 0.05$). For each species, points with the same letter are not significantly different. Data analysis was done for all species collectively.

Figure 49 Effect of sunflower ground shoot on germination and growth of *Cephalaria syriaca.*

Figure 50 Effect of sunflower ground shoot on growth of representative *Cephalaria syriaca* seedlings.

3.3.1.4 Effects on shoot dry weight

Shoot dry weights of *A. fatua* were significantly reduced by two month sunflower ground shoot (Figure 51) at all concentrations. In addition, shoot dry weight of *C. syriaca* at 9 g/pot concentration was significantly reduced compared with the control (illustrated in Figures 49 and 50). No significant differences were observed between the controls and any of the concentrations for *B. napus* and *P. minor*.

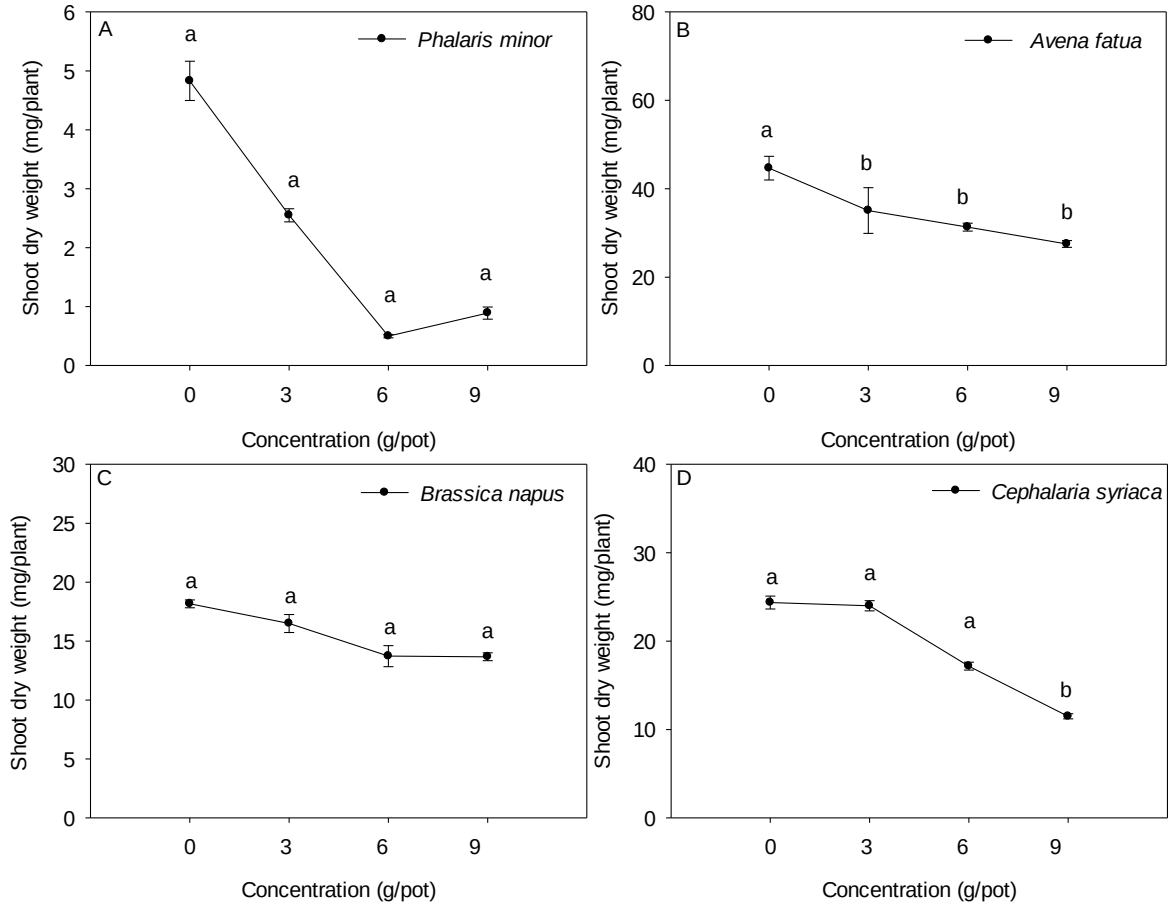


Figure 51 Effects of sunflower ground shoot on shoot dry weight of *Brassica napus*, *Avena fatua*, *Cephalaria syriaca* and *Phalaris minor*.

The results are means of triplicate samples. Error bars represent standard errors. Significant differences between means were calculated based on Tukey's test ($P \leq 0.05$). For each species, points with the same letter are not significantly different. Data analysis was done for all species collectively.

3.3.1.5 Effects on root dry weight

Two month sunflower ground shoot at the highest concentration (9 g/ pot) exhibited significant inhibitory effects on root dry weight of most plant species (Figure 52). Root dry weight of *A. fatua* and *C. syriaca* was significantly reduced at all concentrations of two month sunflower ground shoot. No significant difference was recorded for *P. minor* (illustrated in Figure 64).

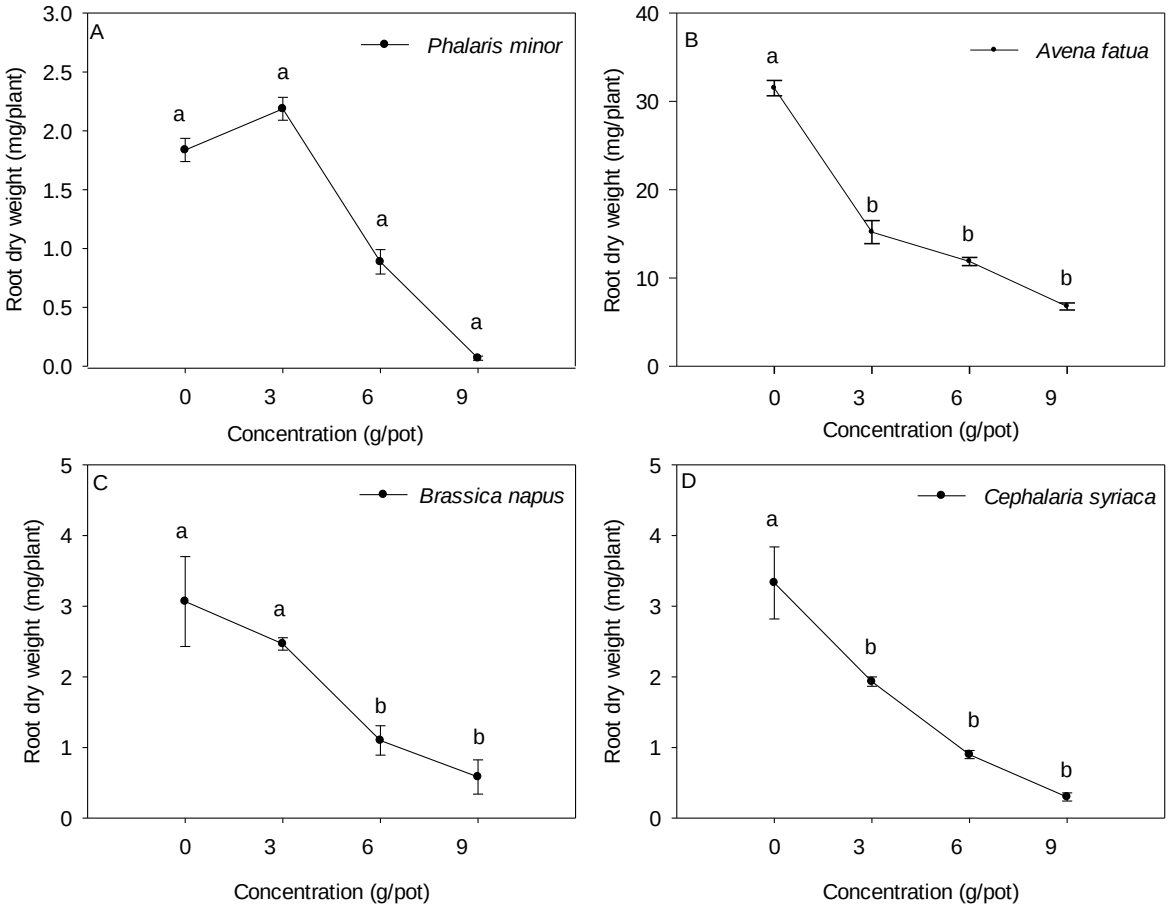


Figure 52 Effect of sunflower ground shoot on root dry weight of *Brassica napus, Avena fatua, Cephalaria syriaca* and *Phalaris minor.*

The results are means of triplicate samples. Error bars represent standard errors. Significant differences between means were calculated based on Tukey's test ($P \leq 0.05$). For each species, points with the same letter are not significantly different. Data analysis was done for all species collectively.

3.3.1.6 Effects of sunflower ground shoot on properties of soil in which rapeseed (*Brassica napus*), wild oat (*Avena fatua*), Syrian Cephalaria (*Cephalaria syriaca*), and littleseed canary grass (*Phalaris minor*) had been grown from seed

3.3.1.6.1 Effect on calcium availability

Two month sunflower ground shoot at 9 g/pot significantly reduced calcium availability of soil in which *A. fatua* or *P. minor* was grown (Figure 53). There was no significant effect for the other species.

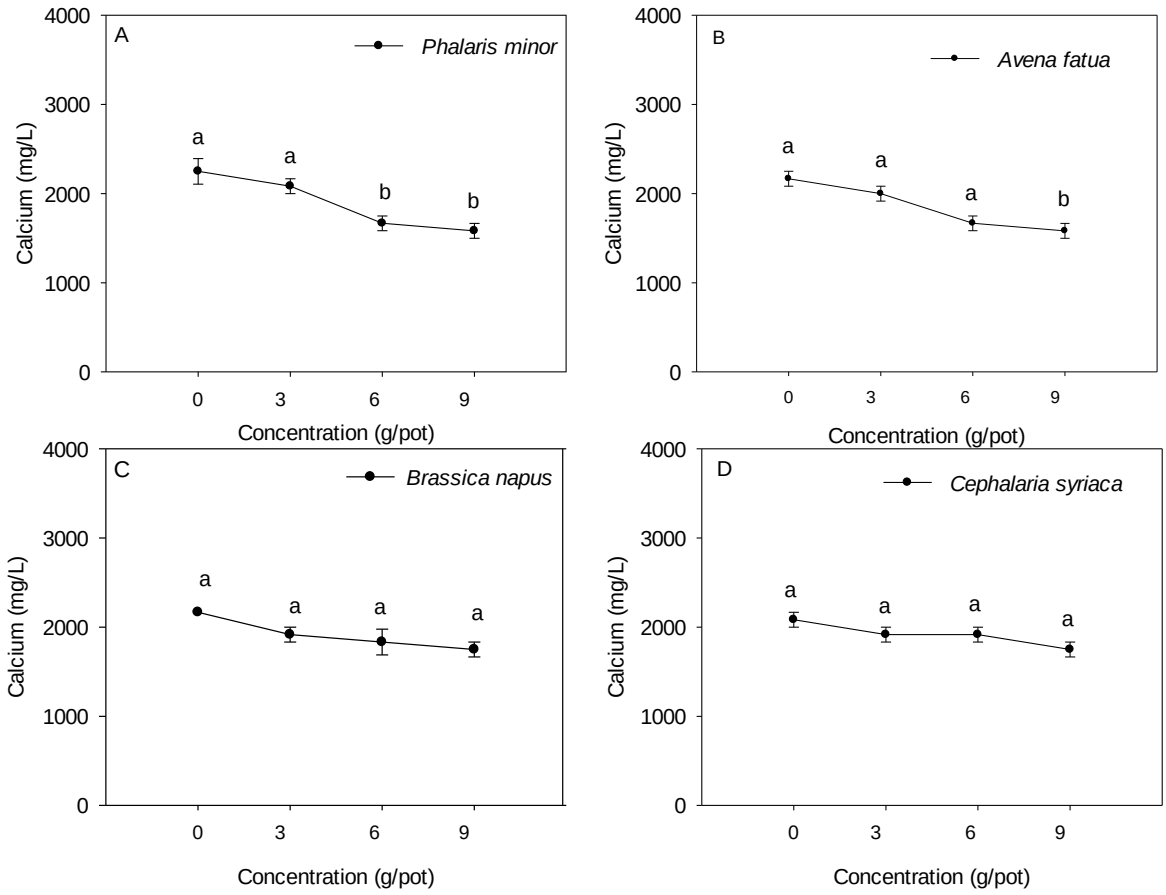


Figure 53 Effect of sunflower ground shoot on calcium availability in soil of *Brassica napus*, *Avena fatua*, *Cephalaria syriaca* and *Phalaris minor*.

The results are means of triplicate samples. Calcium level in the soil expressed as mg/L. Error bars represent standard errors. Significant differences between means were calculated based on Tukey's test ($P \leq 0.05$). For each species, points with the same letter are not significantly different. Data analysis was done for all species collectively.

3.3.1.6.2 Effect on magnesium availability

Figure 54 shows the effect of two month sunflower ground shoot on magnesium availability in soil. There was no significant difference between the control and other concentrations in any species.

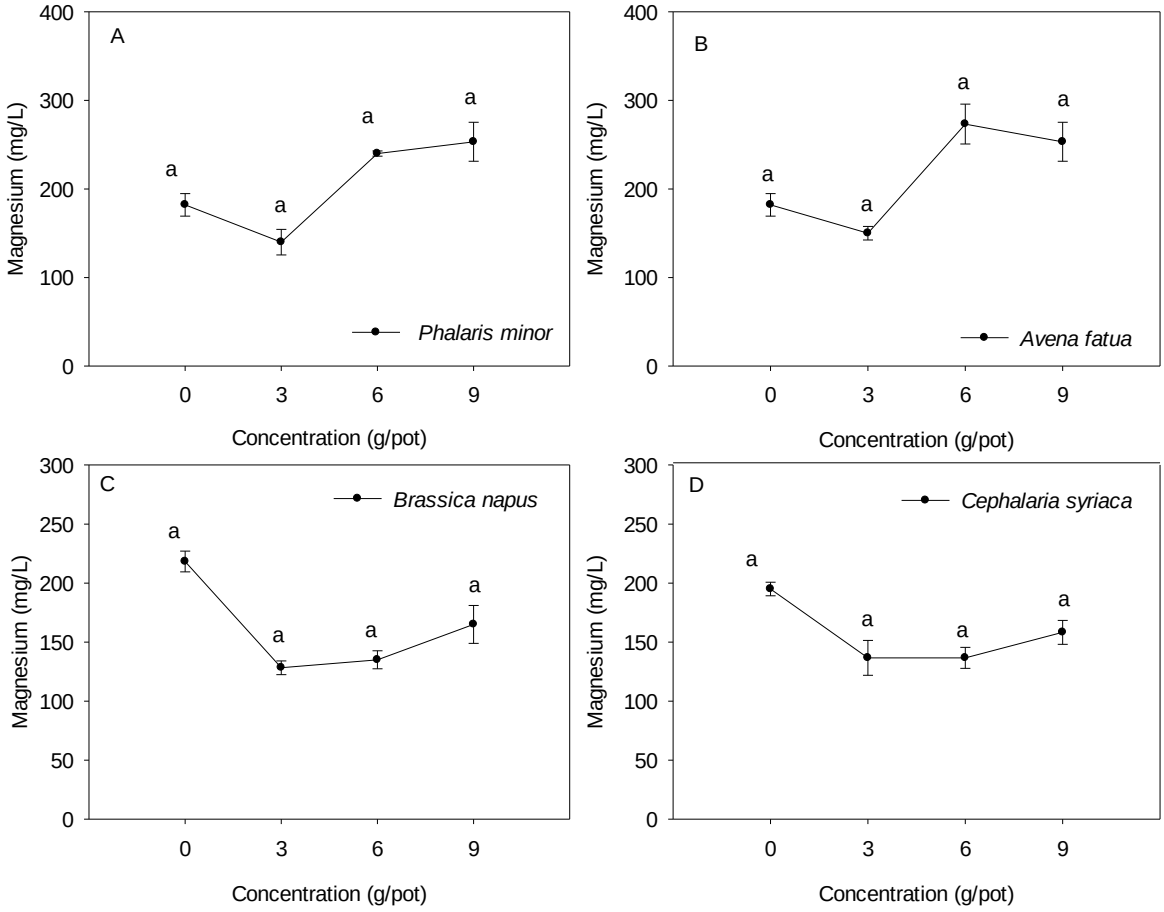


Figure 54 Effect of sunflower ground shoot on magnesium availability in soil of *Brassica napus, Avena fatua*, *Cephalaria syriaca* and *Phalaris minor.*

The results are means of triplicate samples. Magnesium level in the soil expressed as mg/L. Error bars represent standard errors. Significant differences between means were calculated based on Tukey's test ($P \leq 0.05$). For each species, points with the same letter are not significantly different. Data analysis was done for all species collectively.

3.3.1.6.3 Effect on potassium availability

Two month sunflower ground shoot applied at concentrations of 3, 6, and 9 g/pot to *A. fatua* and *P. minor* significantly increased potassium availability in soil (Figure 55). Moreover, potassium availability was significantly increased by sunflower ground shoot applied at 9 g/pot to *B. napus* and *C. syriaca.*

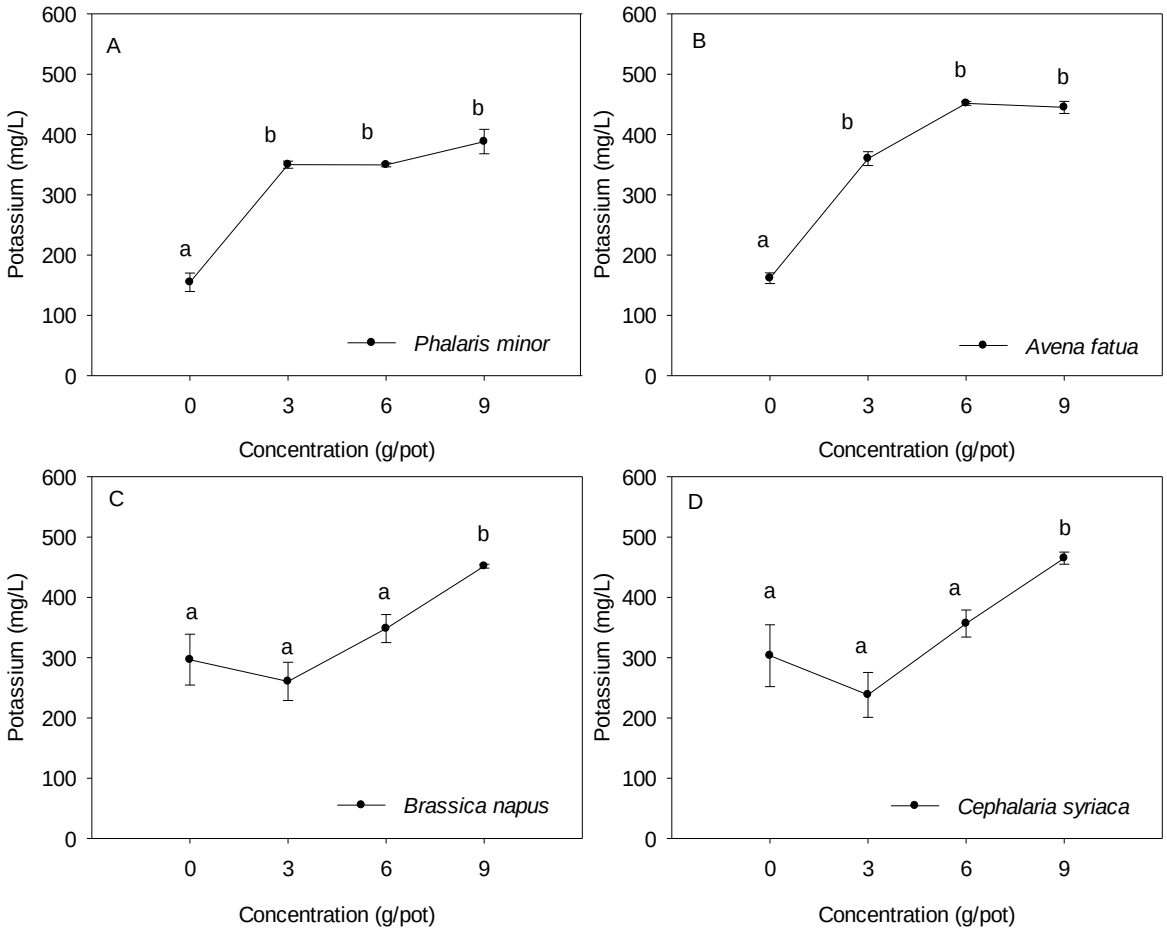


Figure 55 Effect of sunflower ground shoot on potassium availability in soil of *Brassica napus*, *Avena fatua*, *Cephalaria syriaca* and *Phalaris minor.*

The results are means of triplicate samples. Potassium level in the soil expressed as mg/L. Error bars represent standard errors. Significant differences between means were calculated based on Tukey's test ($P \leq 0.05$). For each species, points with the same letter are not significantly different. Data analysis was done for all species collectively.

3.3.1.6.4 Effect on soil pH

Two month sunflower ground shoot at a concentration of 9 g/pot significantly decreased pH in the soil of all plant species (Figure 56). Incorporating ground shoot (6 g/pot) with soil for *A. fatua* and *P. minor* significantly decreased pH in the soil. No significant difference was recorded between the control and treatments with 3 g/pot in any species.

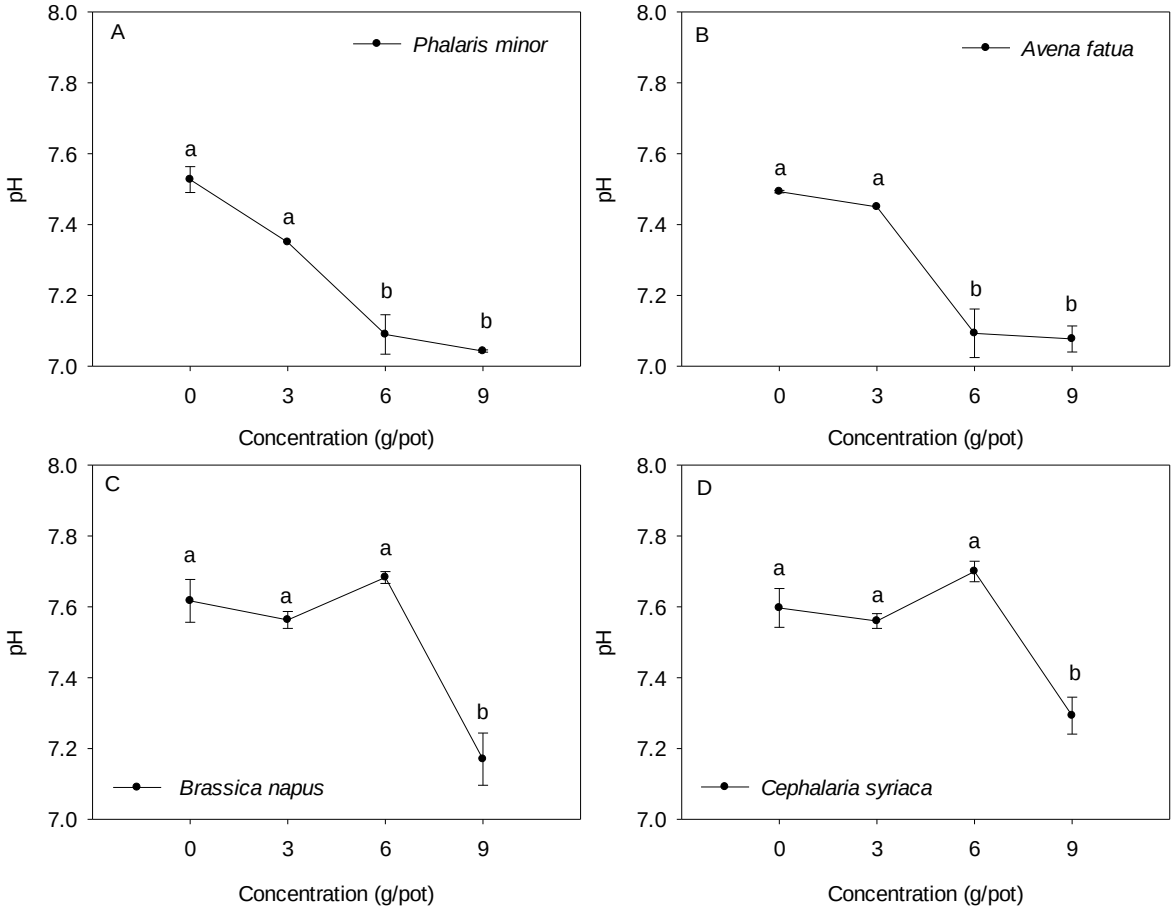


Figure 56 Effect of sunflower ground shoot on pH in soil of *Brassica napus, Avena fatua, Cephalaria syriaca* and *Phalaris minor.*

The results are means of triplicate samples. Error bars represent standard errors. Significant differences between means were calculated based on Tukey's test ($P \leq 0.05$). For each species, points with the same letter are not significantly different. Data analysis was done for all species collectively.

3.3.1.6.5 Effect on soil electrical conductivity (EC)

Two month old sunflower ground shoot at concentrations of 6 and 9 g/pot significantly reduced electrical conductivity of soil planted with *B. napus, A. fatua, C. syriaca* and *P. minor* (Figure 57). However, no significant difference was recorded between control soil and soil with 3 g/pot except for *C. syriaca*.

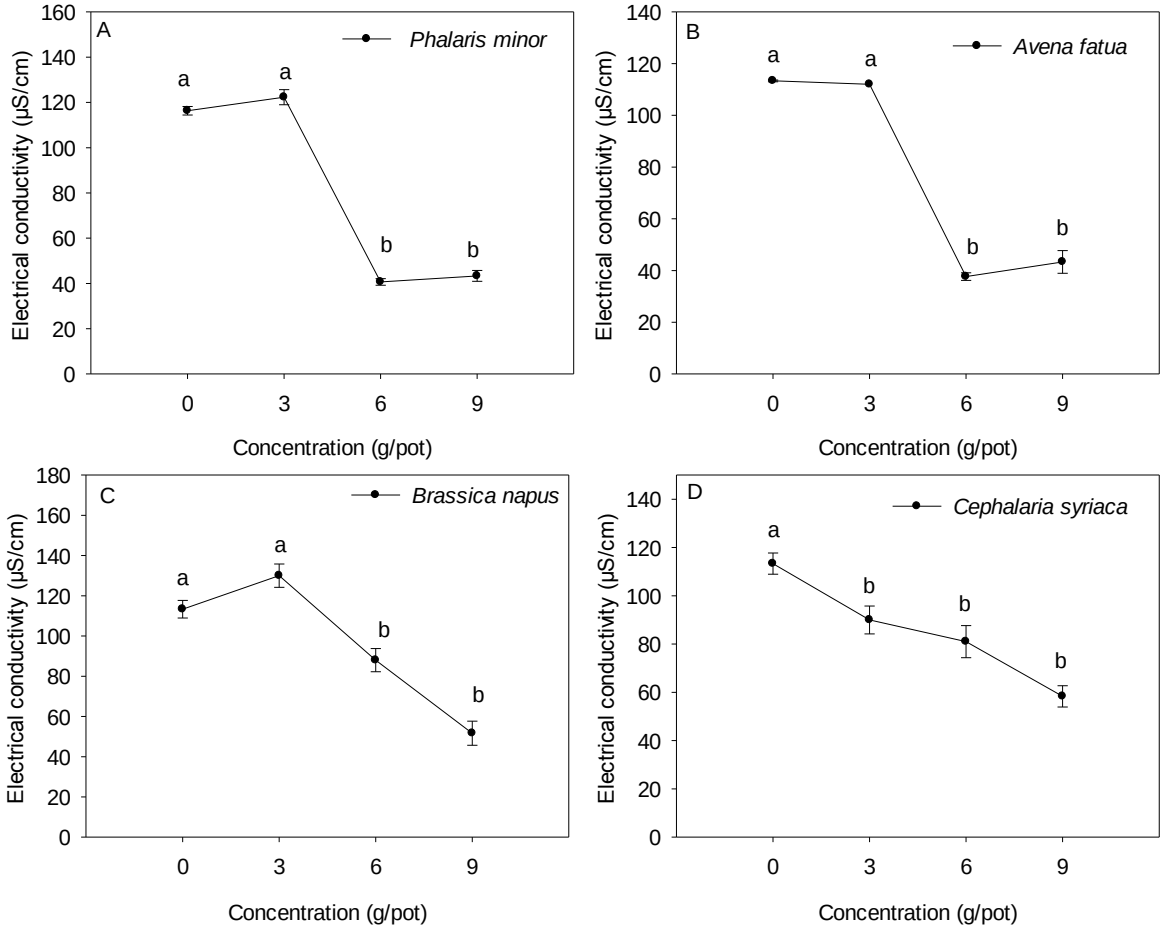


Figure 57 Effect of sunflower ground shoot on electrical conductivity in soil of *Brassica napus, Avena fatua, Cephalaria syriaca* and *Phalaris minor*.

The results are means of triplicate samples. Error bars represent standard errors. Significant differences between means were calculated based on Tukey's test ($P \leq 0.05$). For each species, points with the same letter are not significantly different. Data analysis was done for all species collectively.

3.3.1.7 Effect of sunflower ground shoot on chlorophyll a and chlorophyll b content of rapeseed (*Brassica napus*), wild oat (*Avena fatua*), Syrian Cephalaria (*Cephalaria syriaca*), and littleseed canary grass (*Phalaris minor*)

3.3.1.7.1 Effect on Chlorophyll a

Chlorophyll a content did not differ significantly between control plants and plants treated with any concentration of sunflower ground shoot (Figure 58).

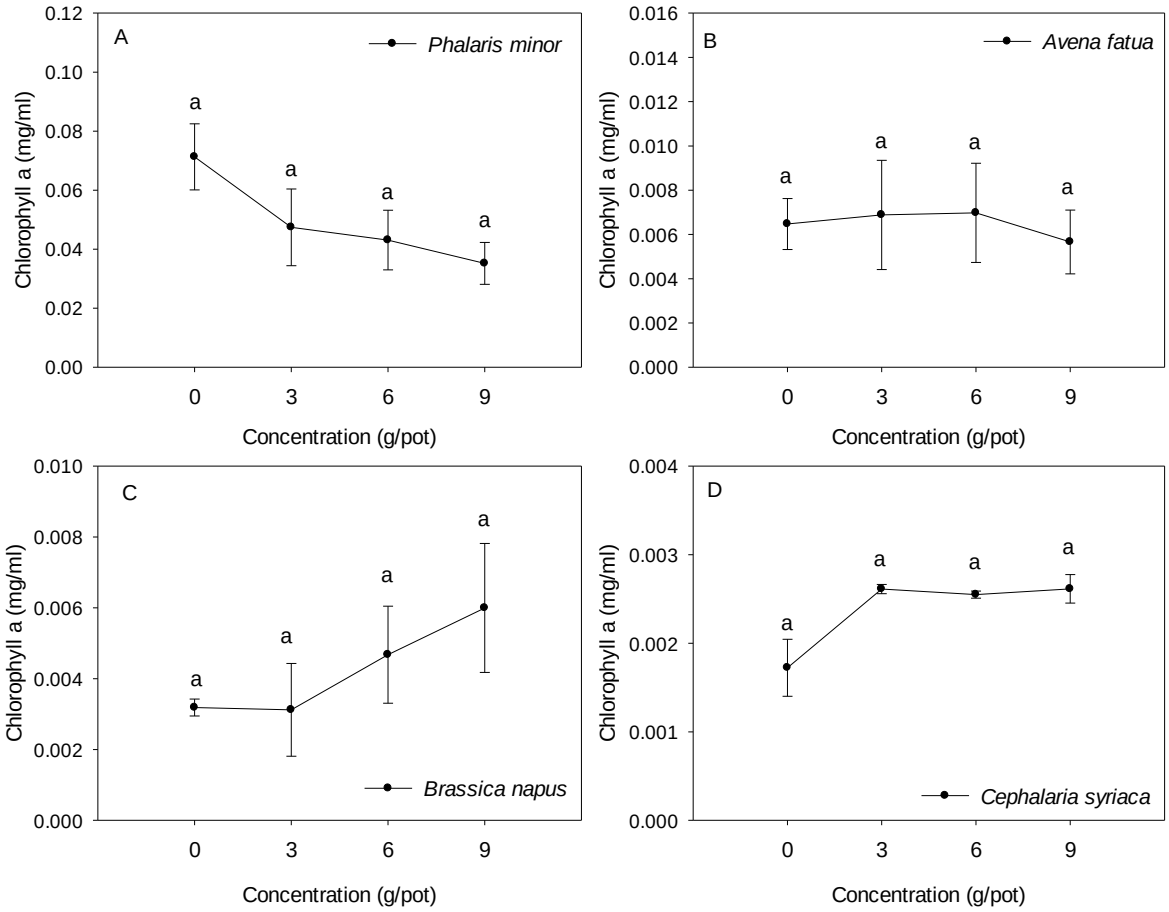


Figure 58 Effect of sunflower ground shoot on chlorophyll a of *Brassica napus*, *Avena fatua*, *Cephalaria syriaca* and *Phalaris minor*.

The results are means of triplicate samples. Error bars represent standard errors. Significant differences between means were calculated based on Tukey's test ($P \leq 0.05$). For each species, points with the same letter are not significantly different. Data analysis was done for all species collectively.

3.3.1.7.2 Effect on chlorophyll b

Analysis of variance showed that at ground shoot concentrations of 6 and 9 g/pot, chlorophyll b was significantly reduced in comparison with the control in *P. minor*. No significant difference was recorded for *B. napus* and *C. syriaca* (Figure 59). There was an increase in chlorophyll b concentration for *A. fatua* with 6 g/pot.

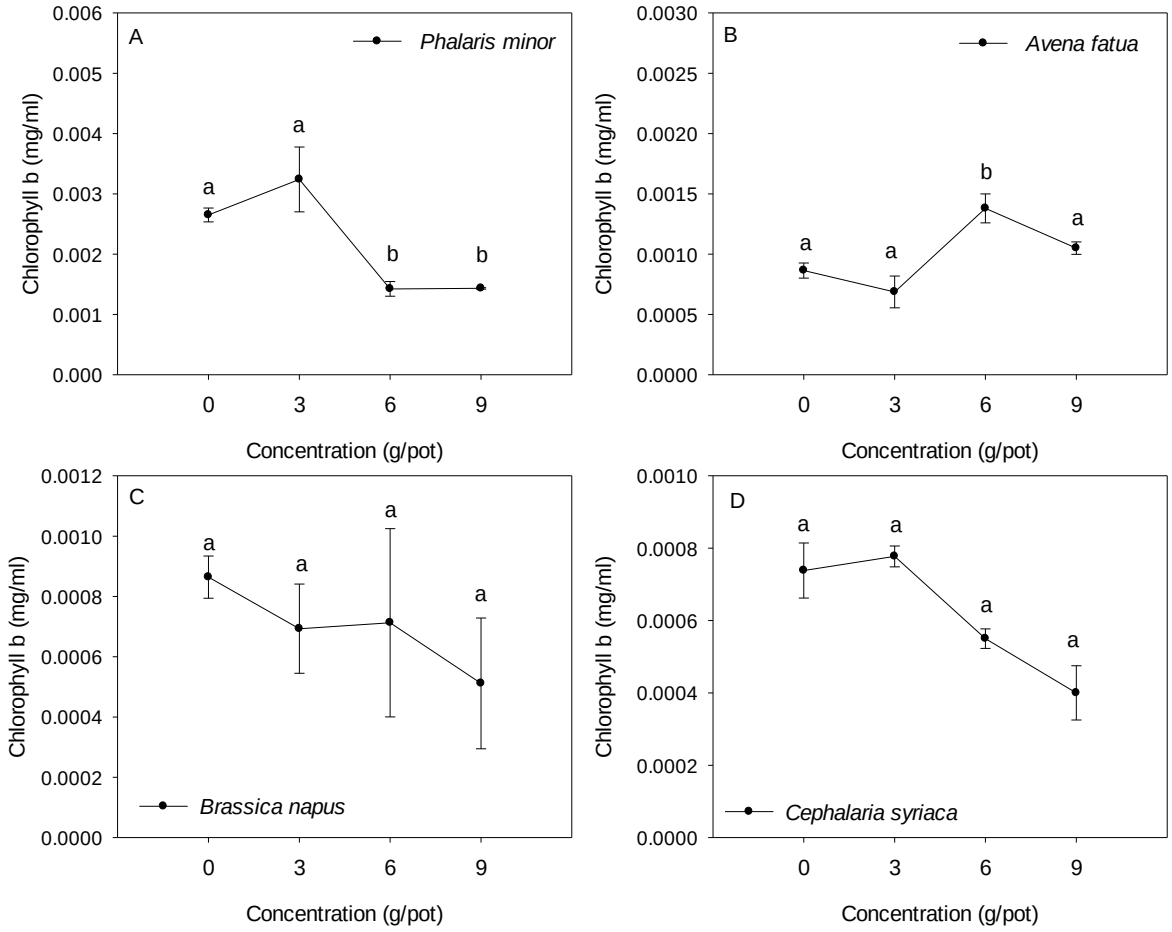


Figure 59 Effect of sunflower ground shoot on chlorophyll b of *Brassica napus*, *Avena fatua*, *Cephalaria syriaca* and *Phalaris minor*.

The results are means of triplicate samples. Error bars represent standard errors. Significant differences between means were calculated based on Tukey's test ($P \leq 0.05$). For each species, points with the same letter are not significantly different. Data analysis was done for all species collectively.

3.3.2 Effect of sunflower two month shoot aqueous extract on germination and early growth of *B. napus*, *A. fatua, C. syriaca* and *P. minor*

3.3.2.1 Effect on seed germination

Figure 60 shows that there were no significant differences in seed germination between the control and any of the treatments (3%, 6% and 9% aqueous shoot extract) for *B. napus*, *A. fatua*, *C. syriaca* and *P. minor* (illustrated in Figure 63).

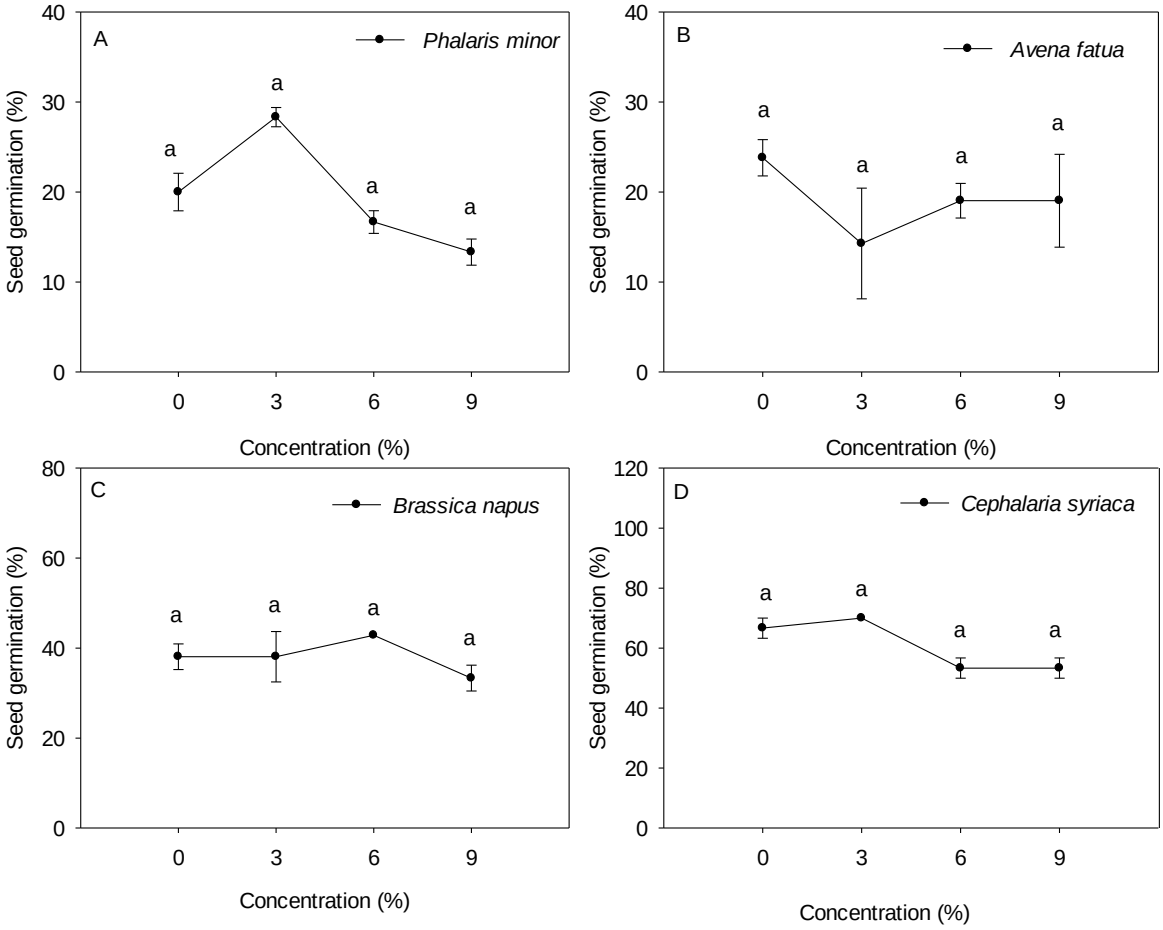


Figure 60 Effect of sunflower aqueous shoot extract on seed germination of *Brassica napus*, *Avena fatua*, *Cephalaria syriaca* and *Phalaris minor*.

The results are means of triplicate samples. Error bars represent standard errors. Significant differences between means were calculated based on Tukey's test ($P \leq 0.05$). For each species, points with the same letter are not significantly different. Data analysis was done for all species collectively.

3.3.2.2 Effect on shoot length

Two month old sunflower shoot aqueous extract (9%) significantly increased shoot length in *B. napus* and *A. fatua* (Figure 61). In contrast, there were no significant differences between controls and other concentrations in *C. syriaca* and *P. minor* (illustrated in Figure 64).

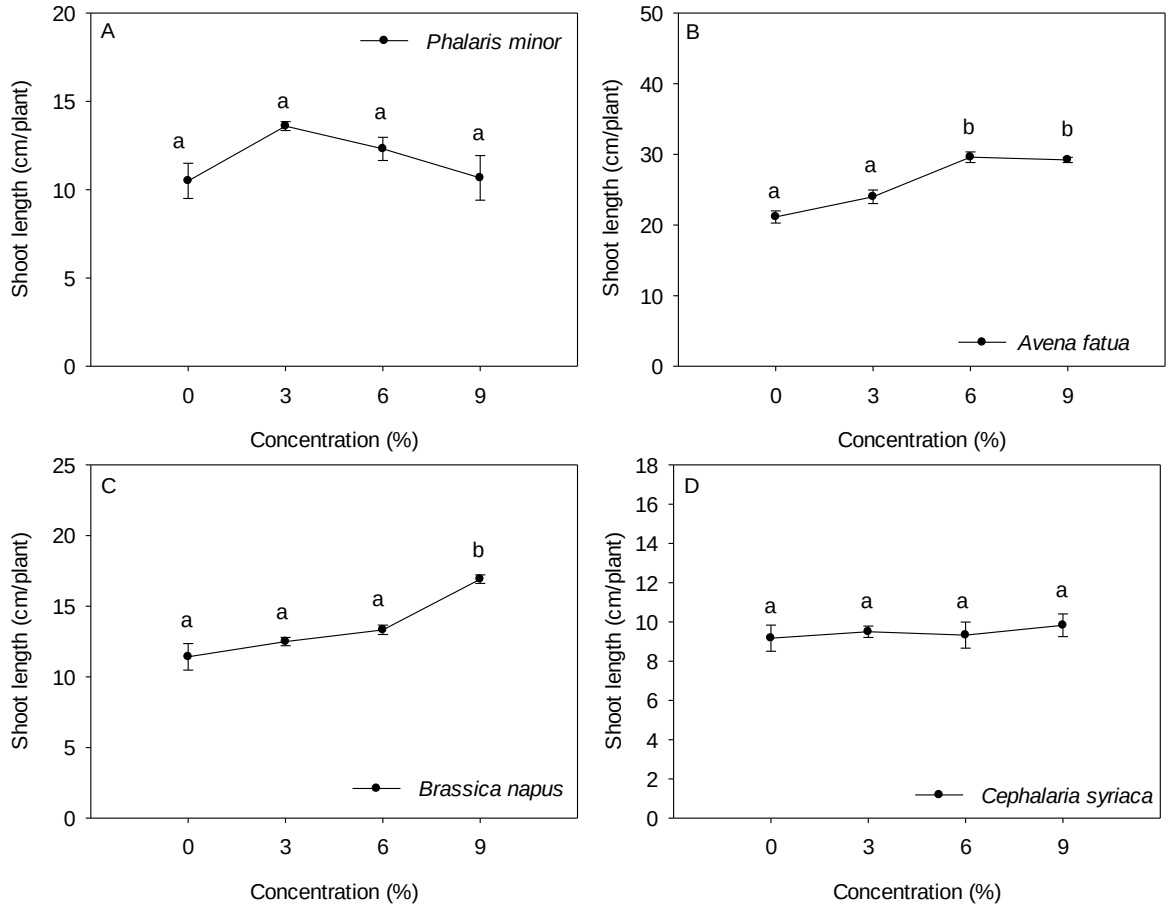


Figure 61 Effect of sunflower aqueous shoot extract on shoot length of *Brassica napus, Avena fatua, Cephalaria syriaca* and *Phalaris minor.*

The results are means of triplicate samples. Error bars represent standard errors. Significant differences between means were calculated based on Tukey's test ($P \leq 0.05$). For each species, points with the same letter are not significantly different. Data analysis was done for all species collectively.

3.3.2.3 Effect on root length

Two month sunflower shoot aqueous extract (3%, 6% and 9%) did not significantly affect root length of *A. fatua, B. napus* and *C. syriaca* (Figure 62). However, the extract did significantly reduce root length of *P. minor* at concentration 6% and 9% (illustrated in Figure 64).

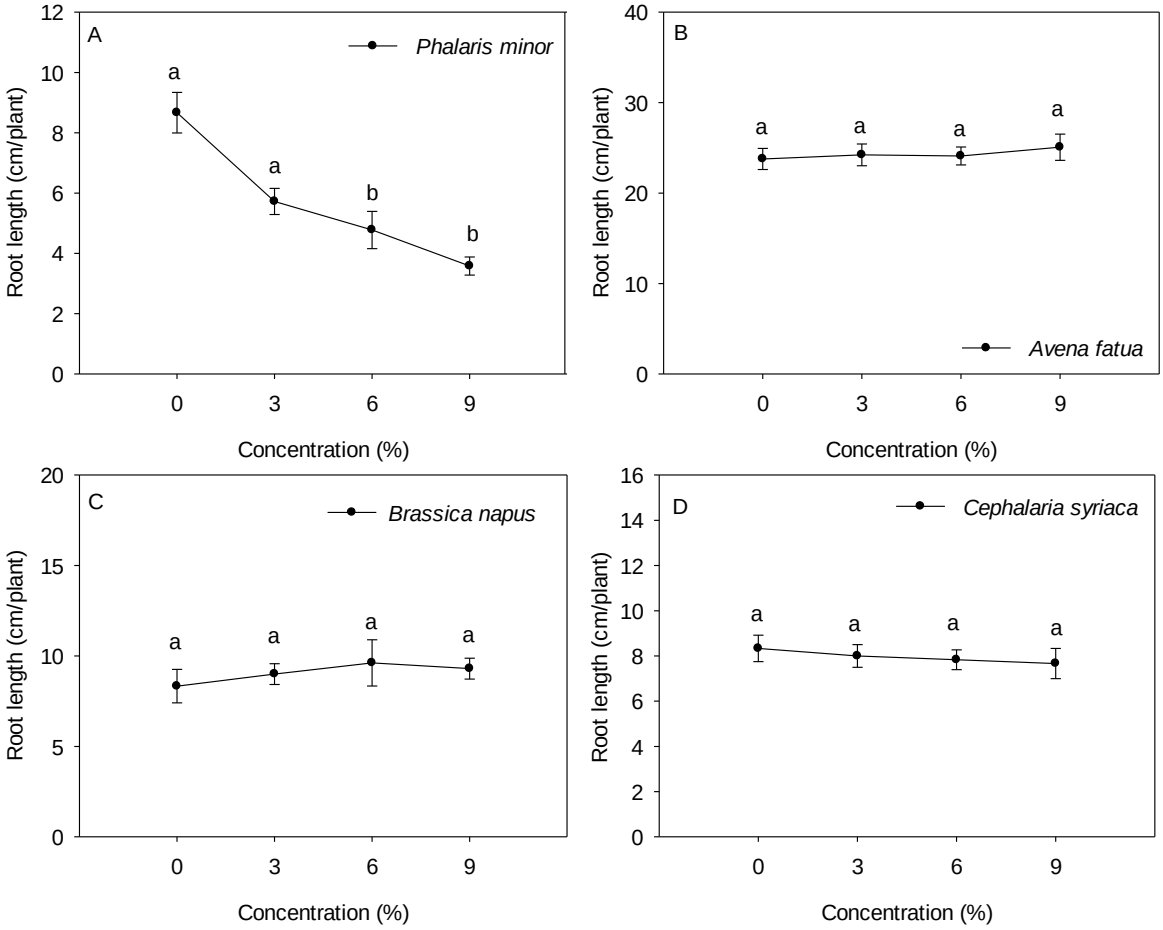


Figure 62 Effect of sunflower aqueous shoot extract on root length of *Brassica napus*, *Avena fatua*, *Cephalaria syriaca* and *Phalaris minor.*

The results are means of triplicate samples. Error bars represent standard errors. Significant differences between means were calculated based on Tukey's test ($P \leq 0.05$). For each species, points with the same letter are not significantly different. Data analysis was done for all species collectively.

Figure 63 Effect of sunflower aqueous shoot extract (3%, 6% and 9%) on germination and growth of *Phalaris minor*.

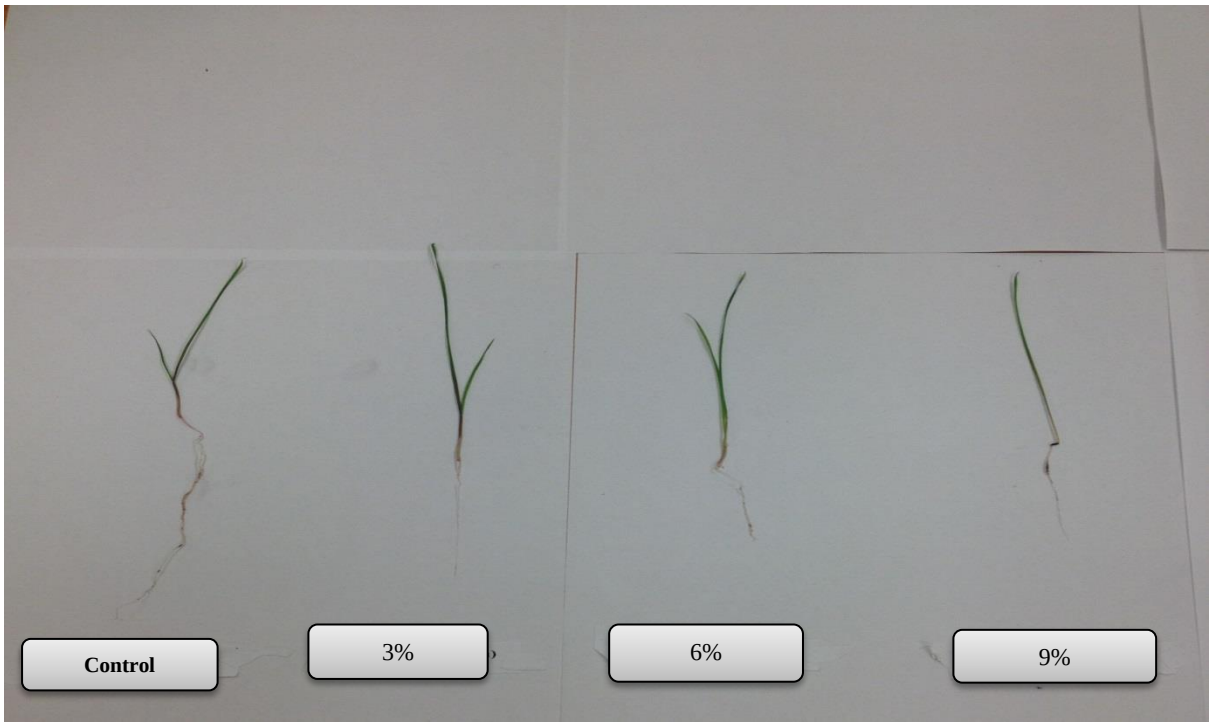


Figure 64 Effect of sunflower aqueous shoot extract (3%, 6%, 9%) on growth of representative *Phalaris minor* seedlings.

3.3.2.4 Effect on shoot dry weight

Figure 65 shows that shoot oven dry weight of *A. fatua* and *B. napus* increased significantly at higher concentrations of two month sunflower shoot aqueous extract. No significant differences from the controls were observed for *P. minor* and *C. syriaca*.

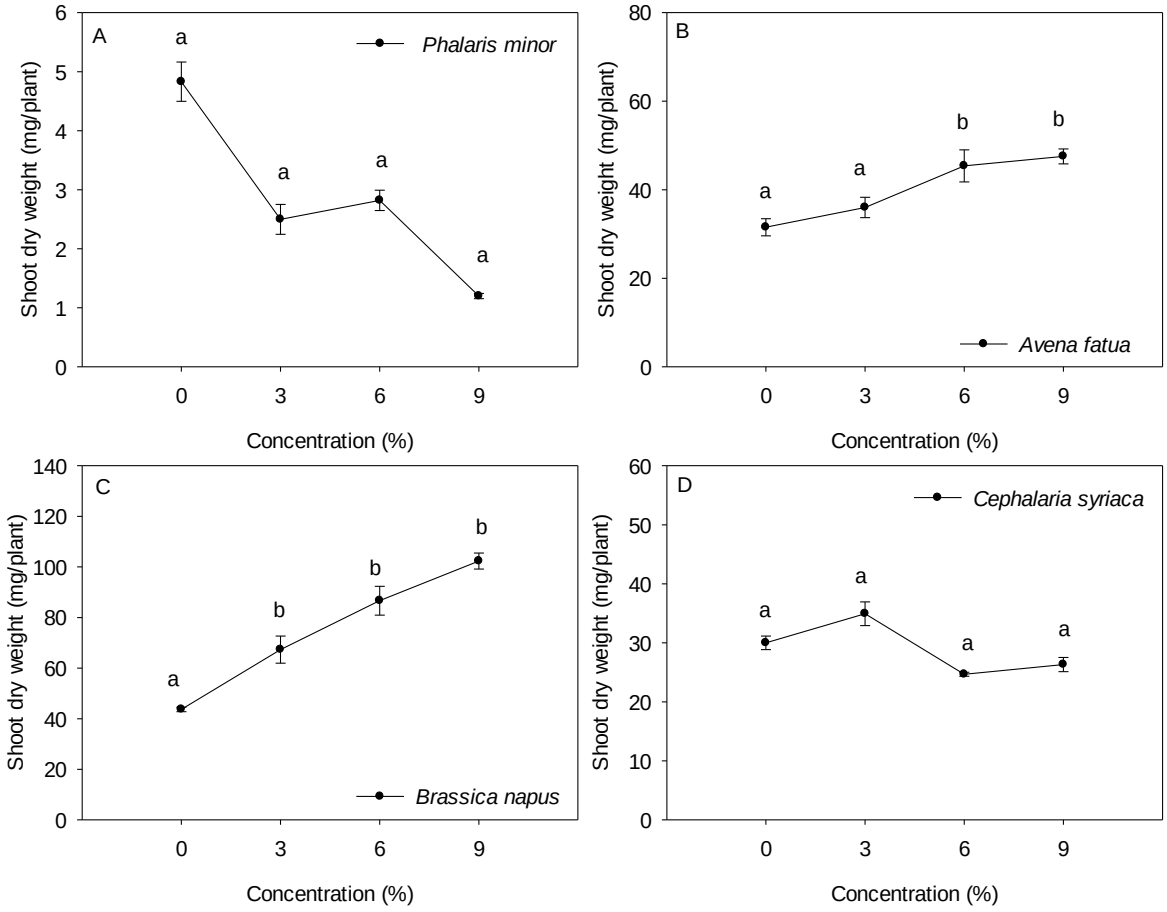


Figure 65 Effect of sunflower aqueous shoot extract on shoot dry weight of *Brassica napus*, *Avena fatua*, *Cephalaria syriaca* and *Phalaris minor*.

The results are means of triplicate samples. Error bars represent standard errors. Significant differences between means were calculated based on Tukey's test ($P \leq 0.05$). For each species, points with the same letter are not significantly different. Data analysis was done for all species collectively.

3.3.2.5 Effect on root dry weight

Figure 66 shows the effect of two month old sunflower shoot aqueous extract on root dry matter of *P. minor, A. fatua, B. napus* and *C. syriaca.* No significant difference was observed between the control and any concentrations of sunflower aqueous extract (3%, 6% and 9%) in *P. minor* and *C. syriaca*. Root dry weight of *B. napus* was significantly promoted at 3% of sunflower aqueous extract. Nevertheless, all tested concentrations of shoot sunflower aqueous extract significantly reduced root dry weight of *A. fatua.*

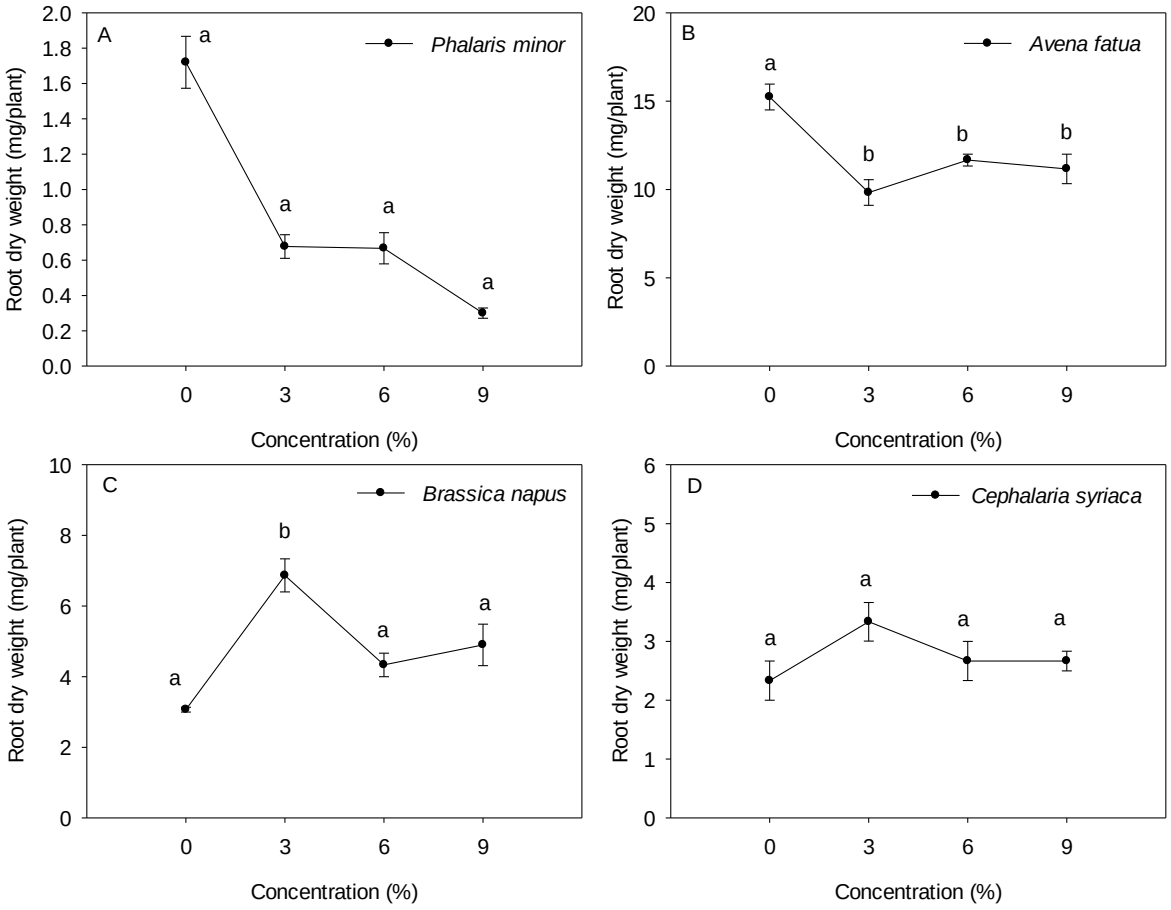


Figure 66 Effect of sunflower aqueous shoot extract on root dry weight of *Brassica napus*, *Avena fatua*, *Cephalaria syriaca* and *Phalaris minor.*

The results are means of triplicate samples. Error bars represent standard errors. Significant differences between means were calculated based on Tukey's test ($P \leq 0.05$). For each species, points with the same letter are not significantly different. Data analysis was done for all species collectively.

3.3.2.6 Effects of sunflower shoot aqueous extract on properties of soil in which rapeseed (*Brassica napus*), wild oat (*Avena fatua*), Syrian Cephalaria (*Cephalaria syriaca*), and littleseed canary grass (*Phalaris minor*) had been grown from seed

3.3.2.6.1 Effect on calcium availability

Figure 67 shows the effect of two month sunflower aqueous extract in a pot experiment on calcium availability in soil of *P. minor*, *A. fatua*, *B. napus* and *C. syriaca*. There was no significant difference between the control and concentrations of 3%, 6% and 9% in any species.

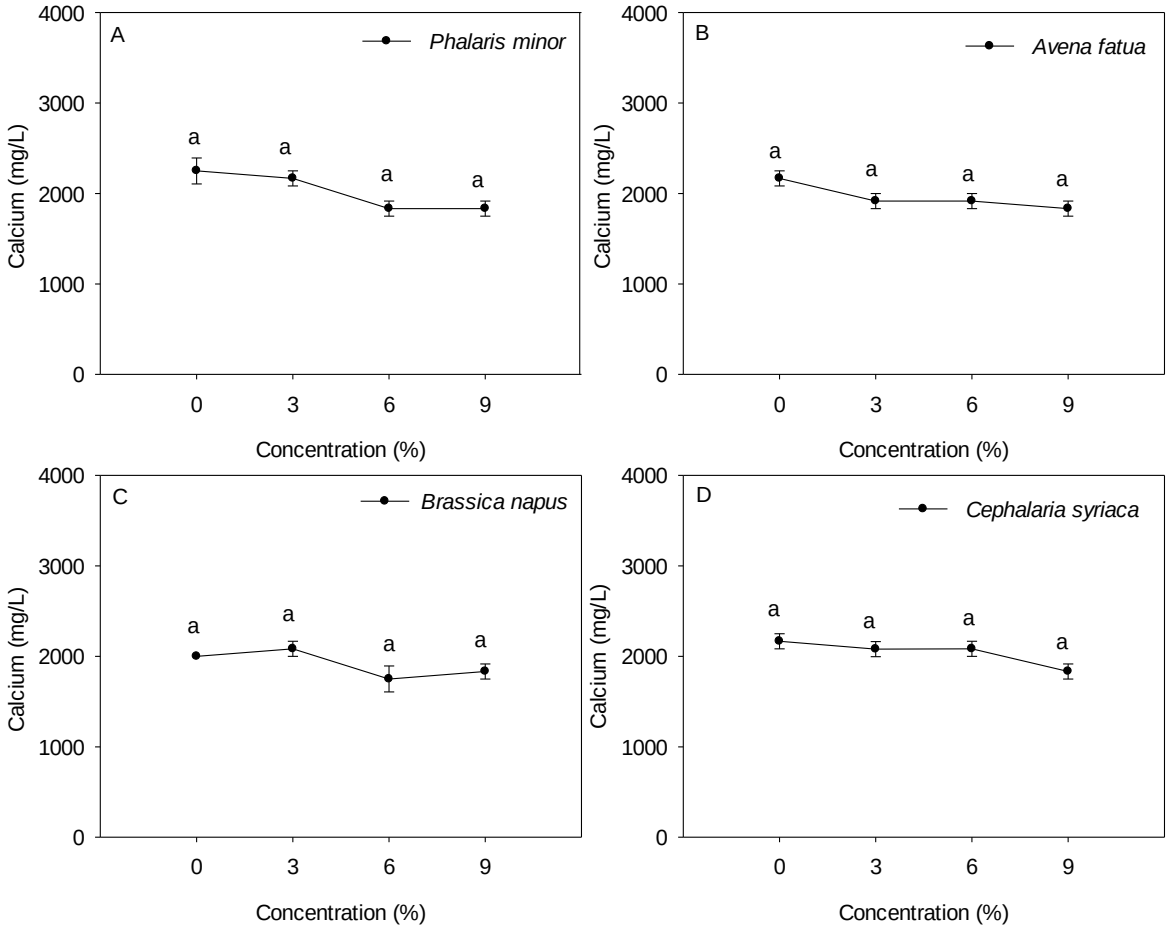


Figure 67 Effect of sunflower aqueous shoot extract on calcium availability in soil of *Brassica napus*, *Avena fatua*, *Cephalaria syriaca* and *Phalaris minor.*

The results are means of triplicate samples. Calcium level in the soil expressed as mg/L. Error bars represent standard errors. Significant differences between means were calculated based on Tukey's test ($P \leq 0.05$). For each species, points with the same letter are not significantly different. Data analysis was done for all species collectively.

3.3.2.6.2 Effect on magnesium availability

Two month sunflower shoot aqueous extract applied to soil at a concentration of 9% significantly decreased the availability of magnesium in *A. fatua* soil (Figure 68). However, there was no significant difference between the control and other treatments for *P. minor, B. napus* and *C. syriaca*.

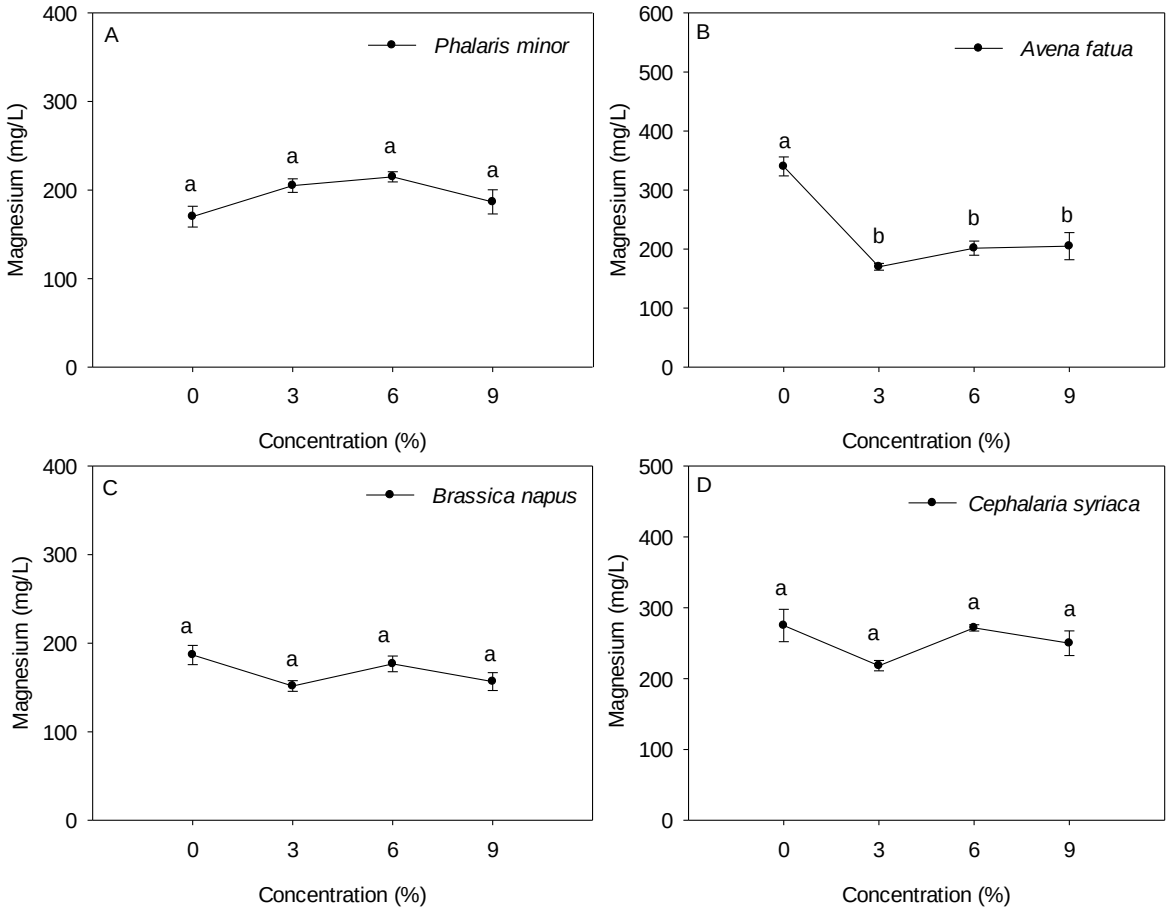


Figure 68 Effect of sunflower aqueous shoot extract on magnesium availability in soil of *Brassica napus, Avena fatua, Cephalaria syriaca* and *Phalaris minor*.

The results are means of triplicate samples. Magnesium level in the soil expressed as mg/L. Error bars represent standard errors. Significant differences between means were calculated based on Tukey's test ($P \leq 0.05$). For each species, points with the same letter are not significantly different. Data analysis was done for all species collectively.

3.3.2.6.3 Effect on potassium availability

Figure 69 shows that two month old sunflower shoot aqueous extract significantly increased the availability of potassium in soil of *P. minor* at a concentration of 9%. However, there was no significant difference between soil in the control and other treatments for *B. napus*, *A. fatua*, and *C. syriaca.*

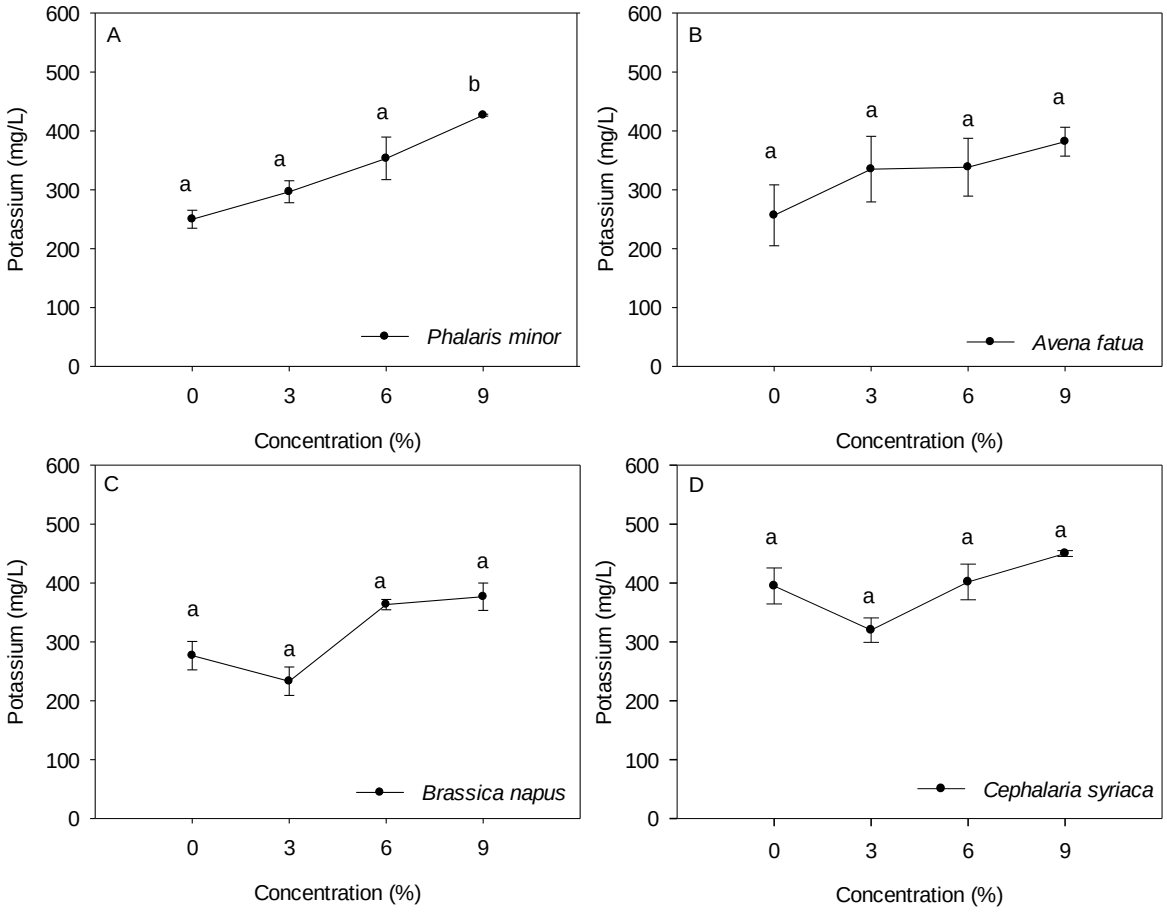


Figure 69 Effect of sunflower aqueous shoot extract on potassium availability in soil of *Brassica napus*, *Avena fatua*, *Cephalaria syriaca* and *Phalaris minor.*

The results are means of triplicate samples. Potassium level in the soil expressed as mg/L. Error bars represent standard errors. Significant differences between means were calculated based on Tukey's test ($P \leq 0.05$). For each species, points with the same letter are not significantly different. Data analysis was done for all species collectively.

3.3.2.6.4 Effect on soil pH

There was no significant effect of two month sunflower shoot aqueous extract on pH of soil in which each species was grown (Figure 70).

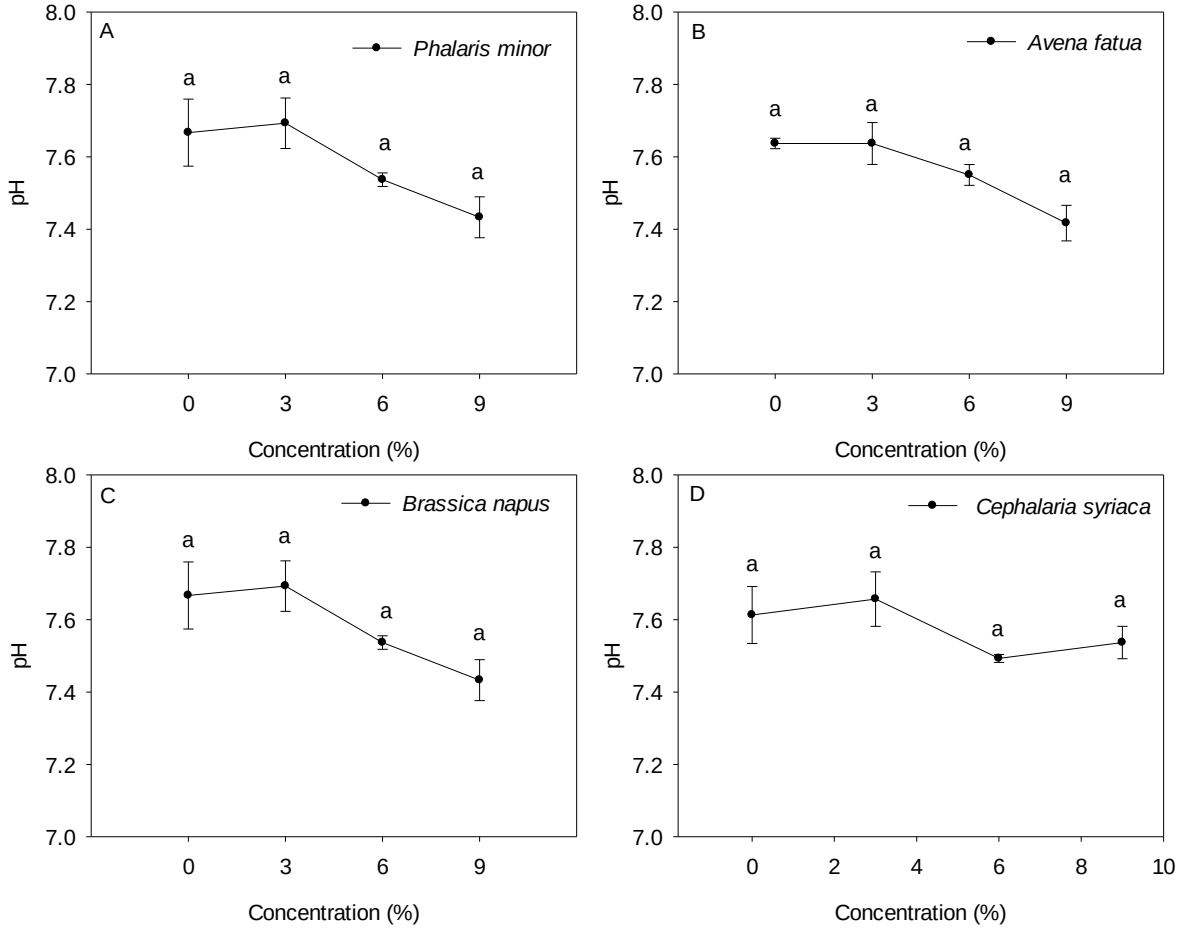


Figure 70 Effect of sunflower aqueous shoot extract on soil pH of *Brassica napus*, *Avena fatua*, *Cephalaria syriaca* and *Phalaris minor*.

The results are means of triplicate samples. Error bars represent standard errors. Significant differences between means were calculated based on Tukey's test ($P \leq 0.05$). For each species, points with the same letter are not significantly different. Data analysis was done for all species collectively.

3.3.2.6.5 Effect on soil electrical conductivity (EC)

Effects of different concentrations of two month old sunflower shoot aqueous extract on EC of soil are shown in Figure 71. Sunflower shoot aqueous extract significantly reduced EC at higher concentrations in *C. syriaca*. Electrical conductivity was significantly reduced by all three concentrations (3, 6, and 9%) in *P. minor* and *B. napus*.

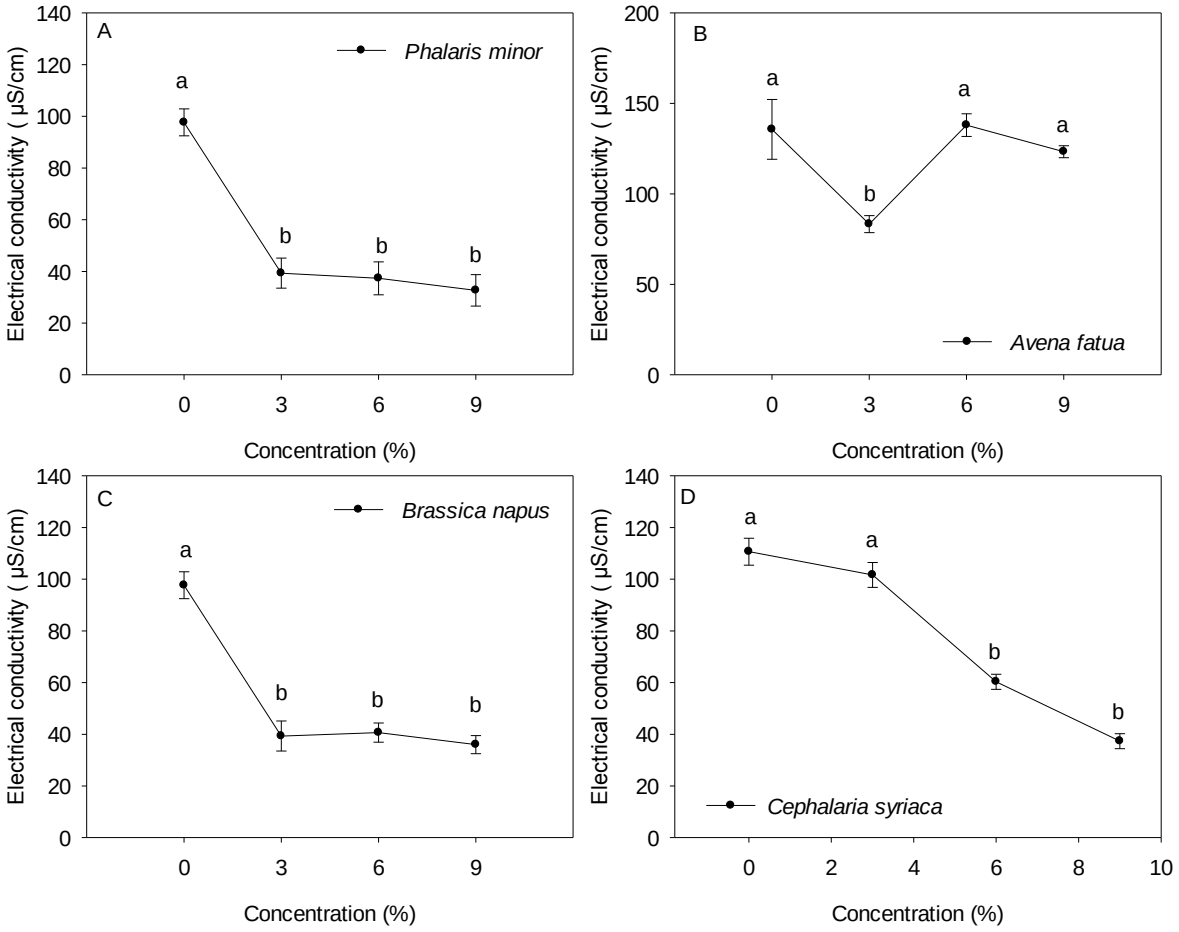


Figure 71 Effect of sunflower aqueous shoot extract on soil electrical conductivity of *Brassica napus*, *Avena fatua*, *Cephalaria syriaca* and *Phalaris minor.*

The results are means of triplicate samples. Error bars represent standard errors. Significant differences between means were calculated based on Tukey's test ($P \leq 0.05$). For each species, points with the same letter are not significantly different. Data analysis was done for all species collectively.

3.3.2.7 Effect of sunflower shoot aqueous extract on chlorophyll a and chlorophyll b content of rapeseed (*Brassica napus*), wild oat (*Avena fatua*), Syrian Cephalaria (*Cephalaria syriaca*), and littleseed canary grass (*Phalaris minor*)

3.3.2.7.1 Effect on chlorophyll a

Figure 72 shows that there was no significant effect of two month sunflower shoot aqueous extract at any concentration on chlorophyll a of leaves of any species.

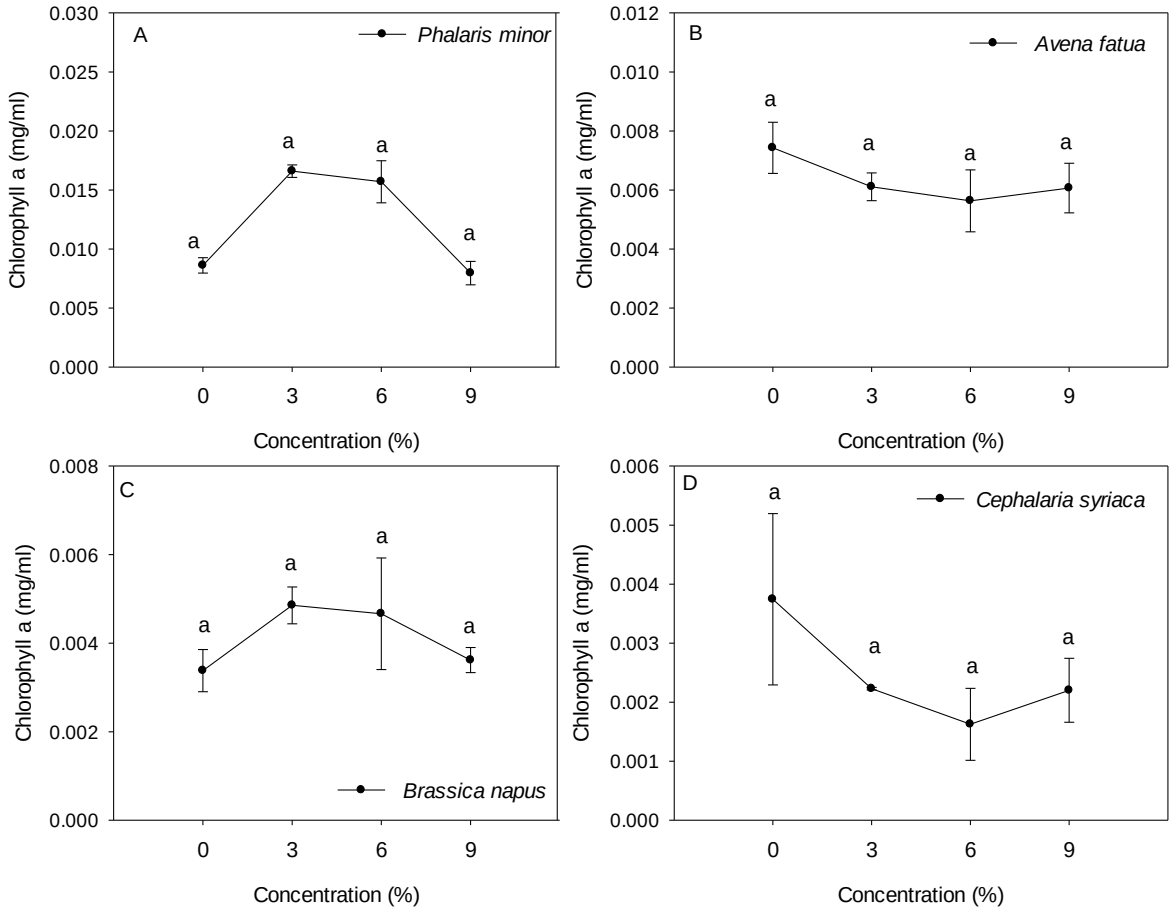


Figure 72 Effect of sunflower aqueous shoot extract on chlorophyll a content of *Brassica napus*, *Avena fatua*, *Cephalaria syriaca* and *Phalaris minor*.

The results are means of triplicate samples. Error bars represent standard errors. Significant differences between means were calculated based on Tukey's test ($P \leq 0.05$). For each species, points with the same letter are not significantly different. Data analysis was done for all species collectively.

3.3.2.7.2 Effect on chlorophyll b

Chlorophyll b content of *C. syriaca* at 3, 6 g/pot concentration was significantly reduced compared with the control. There was no significant effect of two month sunflower shoots aqueous extract on chlorophyll b content in *P. minor, A. fatua* and *B. napus* at concentration (3, 6, 9 g/pot) (Figure 73).

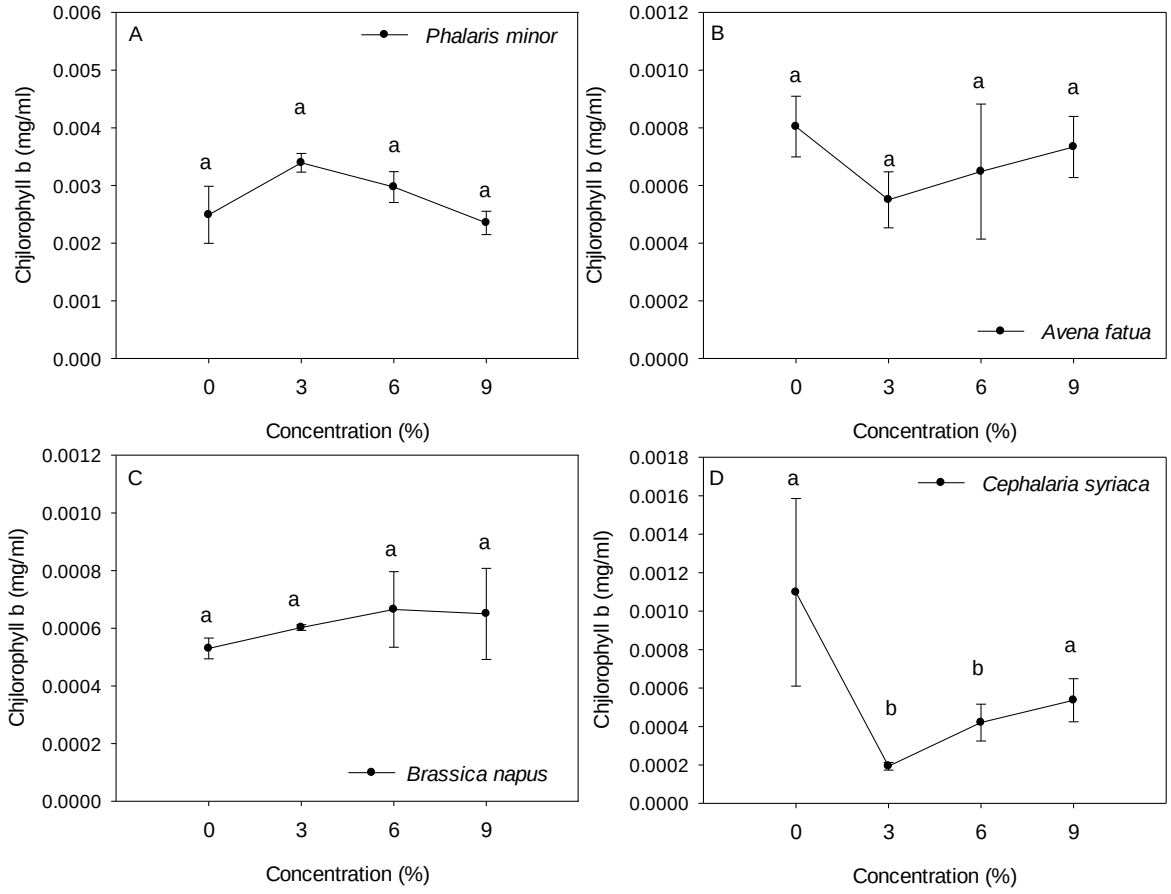


Figure 73 Effect of sunflower aqueous shoot extract on chlorophyll b of *Brassica napus*, *Avena fatua*, *Cephalaria syriaca* and *Phalaris minor*.

The results are means of triplicate samples. Error bars represent standard errors. Significant differences between means were calculated based on Tukey's test ($P \leq 0.05$). For each species, points with the same letter are not significantly different. Data analysis was done for all species collectively.

Experiment 4

The aim of this experiment was to identify and quantify phenolic compounds present in sunflower shoot and root extracts and investigate the effects of individual phenolic compounds on seed germination and seedling growth of *Brassica napus*, *Cephalaria syriaca*, *Triticum aestivum* and *Secale cereale.*

3.4.1 Identification and quantification of phenolic compounds present in sunflower shoot and root extracts from plants at different growth stages (one week, one month, two month and mature stage)

High performance liquid chromatography (HPLC) was operated for identification of phenolic compounds present in sunflower shoot and root samples and determination of their concentrations. In the present study, twelve phenolic compounds were identified from sunflower shoot extracts at different growth stages. The highest concentration of total phenolic compounds was found in two month sunflower shoot extract, in which chlorogenic acid was the main phenolic acid. However, the lowest concentration of total phenolics was observed in sunflower one week root extract. Four phenolic compounds were identified from sunflower root extracts at different growth stages (syringic acid, protocatechuic acid, 4-hydroxybenzoic acid, and ferulic acid). Five phenolic compounds were identified from sunflower one week shoot extract (protocatechuic acid, catechol, ferulic acid, caffeic acid and trans-cinnamic acid). Twelve phenolic compounds were identified and quantified from sunflower shoot one month, two month and mature stage extracts (gallic acid, syringic acid, vanillic acid, protocatechuic acid, catechol, 4-hydroxybenzoic acid, p-coumaric acid, sinapic acid, ferulic acid, caffeic acid, chlorogenic acid, trans-cinnamic acid) (Table 4). Based on the previous studies (Ghafar *et al.* 2001; Alsaadawi *et al.* 2012) standards of phenolic compounds were purchased as there are still some known peaks which have not been identified.

Two month sunflower aqueous shoot extract had the highest concentration of total phenolic acids compared with other growth stages (Table 5). However, one week sunflower root extract had the lowest concentration of phenolic acids.

Table 4 HPLC analysis of phenolic compounds from sunflower shoot and root extracts at different growth stages. Values are in mg/ml of the extracts.

Phenolic compound	One week root	One month root	Two month root	Mature root	One week shoot	One month shoot	Two month shoot	Mature shoot
Gallic acid	-----	-----	-----	-----	-----	0.0065	0.008	0.0131
Syringic acid	0.0010	0.0039	0.0108	0.0025	-----	0.0054	0.0221	0.0033
Vanillic acid	-----	-----	-----	-----	-----	0.0025	0.00864	0.0186
Protocatechuic acid	0.0013	0.0025	0.0028	0.0015	0.0024	0.0006	0.006	0.0056
Catechol (1,2-dihydroxybenzene)	-----	-----	-----	-----	0.0166	0.0060	0.0026	0.0118
4-Hydroxybenzoic acid	0.0010	0.0225	0.0012	0.0031	-----	0.0021	0.0064	0.0107
p-Coumaric acid	-----	-----	-----	-----	-----	0.0104	0.0191	0.0094
Sinapic acid	-----	-----	-----	-----	-----	0.0132	0.0076	0.0158
Ferulic acid	0.0031	0.0143	0.0115	0.0171	0.0272	0.0205	0.0365	0.0361
Caffeic acid	-----	-----	-----	-----	0.0394	0.0158	0.05	0.0379
Chlorogenic acid	-----	-----	-----	-----	-----	0.0187	0.097	0.0486
Trans-cinnamic acid	-----	-----	-----	-----	0.0015	0.0042	0.002	0.0027
Total Phenolics	0.0064	0.0434	0.0265	0.0244	0.0872	0.1063	0.2759	0.21474

Table 5 Phenolic compounds in sunflower two month shoot extract. Concentrations shown were used for the experiments described in sections 3.4.2 and 3.5.2.

Phenolic compound	Concentration (mg/ml)	Concentration (ppm)	Proportion of total phenolics (%)
Trans-cinnamic acid	0.002	2	0.752
Catechol (1,2-dihydroxybenzene)	0.0026	2.6	0.977
Protocatechuic acid	0.006	6	2.256
4-Hydroxybenzoic acid	0.0064	6.4	2.406
Sinapic acid	0.0076	7.6	2.857
Gallic acid	0.008	8	3.008
Vanillic acid	0.00864	8.64	3.248
p-Coumaric acid	0.0191	19.1	7.182
Syringic acid	0.0221	22.1	8.310
Ferulic acid	0.0365	36.5	13.724
Caffeic acid	0.05	50	18.801
Chlorogenic acid	0.097	97	36.474
Total Phenolics	0.27594	265.94	100

3.4.2 Effects of phenolic compounds on germination and early growth of *Brassica napus, Cephalaria syriaca, Triticum aestivum* and *Secale cereale*

3.4.2.1 Effect of phenolic compounds on seed germination

Based on Tukey tests, the data shown in Figure 74 shows a combination of phenolic compounds representing the total phenolic compounds isolated from sunflower two month old shoot extract (Table 5) significantly ($P < 0.001$) affected seed germination in *B. napus*, *C. syriaca*, and *T. aestivum*. Furthermore, sinapic acid significantly reduced seed germination percentage of *B. napus* while seed germination of *T. aestivum* was significantly reduced by gallic acid, p-coumaric acid, ferulic acid and caffeic acid. However, there was no significant difference between the control treatment and other phenolic acid treatments (illustrated in Figures 79 and 80).

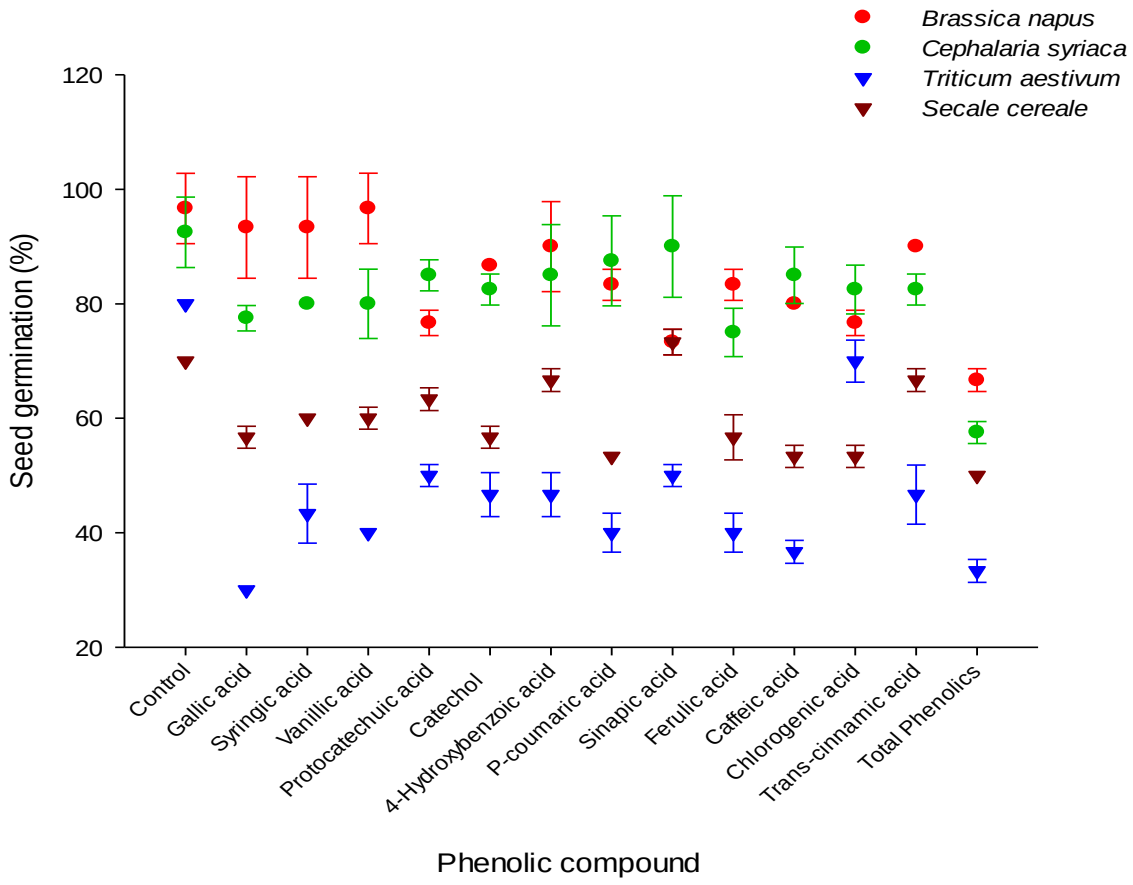


Figure 74 Effects of phenolic compounds on seed germination of *Brassica napus*, *Cephalaria syriaca*, *Triticum aestivum* and *Secale cereale.*

Concentrations of phenolic compounds are shown in Table 5. Control: distilled water. The results are means of triplicate samples. Error bars represent standard errors. Data analysis was done for all species collectively.

3.4.2.2 Effect of phenolic compounds on shoot length

Figure 75 shows the effects of phenolic compounds on shoot lengths of *B. napus*, *C. syriaca*, *T. aestivum* and *S. cereale*. Data analysis indicated that total phenolic compounds had the greatest effect on shoot length of most species. Shoot lengths of *C. syriaca*, *T. aestivum*, and *S. cereale* were significantly reduced by p-coumaric acid, and caffeic acid while chlorogenic acid significantly reduced shoot length in *T. aestivum* and *S. cereale.* Ferulic acid only significantly affected shoot length of *C. syriaca and S. cereale* (illustrated in Figures 79, 80). Moreover, phenolic compounds, gallic acid, vanillic acid, protocatechuic acid, catechol, sinapic acid, 4-hydroxybenzoic acid, and trans-cinnamic acid significantly affected shoot length of *C. syriaca*. In addition, *S. cereale* was significantly affected by syringic acid and sinapic acid ($P < 0.001$). However, in *B. napus*, there was no significant difference between the control and phenolic compounds.

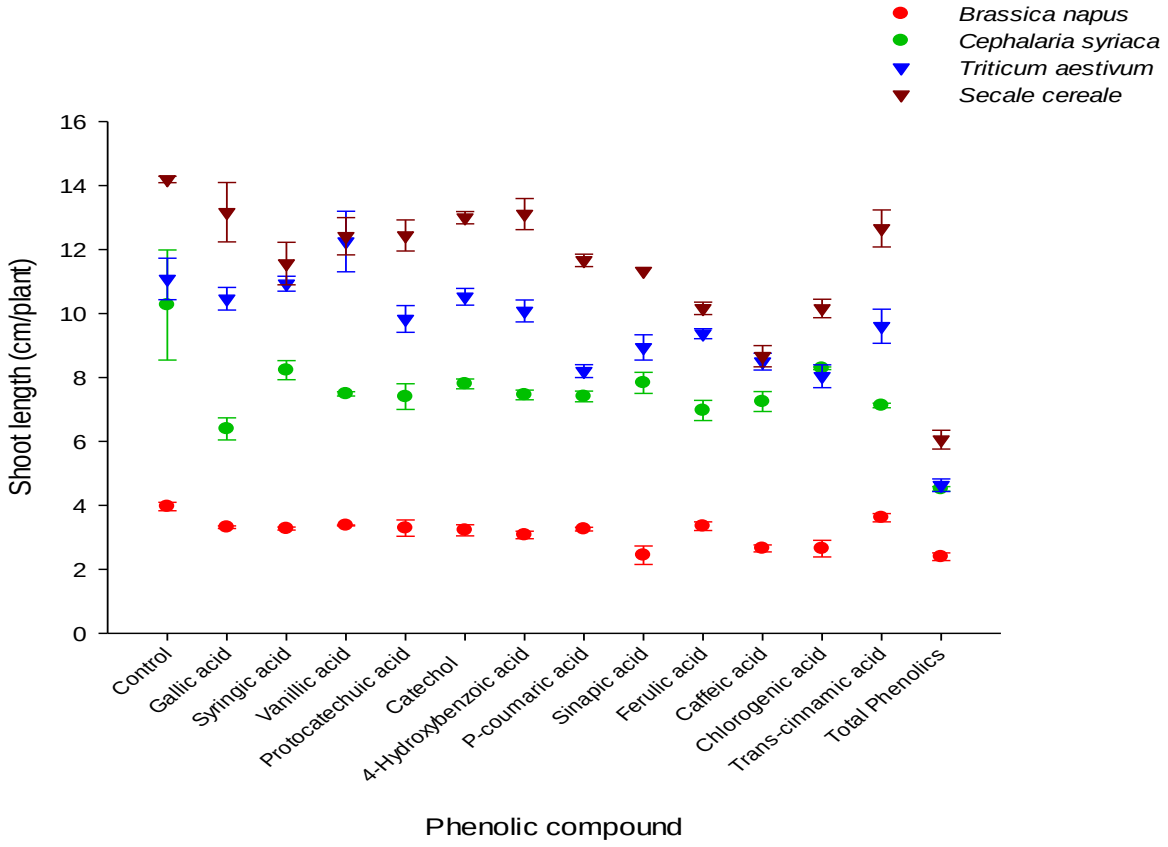


Figure 75 Effects of phenolic compounds on shoot length of *Brassica napus*, *Cephalaria syriaca*, *Triticum aestivum* and *Secale cereale.*

Concentrations of phenolic compounds are shown in Table 5. Control: distilled water. The results are means of triplicate samples. Error bars represent standard errors. Data analysis was done for all species collectively.

3.4.2.3 Effect of phenolic compounds on root length

Total phenolic compounds dramatically reduced root length of all plant species tested (*B. napus*, *C. syriaca*, *T. aestivum* and *S. cereale*). Furthermore, root length of *B. napus* was significantly affected by caffeic acid and chlorogenic acid (Figure 79, 80). In addition, root length of *C. syriaca* was significantly reduced by most phenolic compounds. Sinapic acid and chlorogenic acid significantly ($P < 0.001$) affected root length of *T. aestivum* (Figure 76). Ferulic acid and chlorogenic acid significantly decreased root length of *S. cereale.*

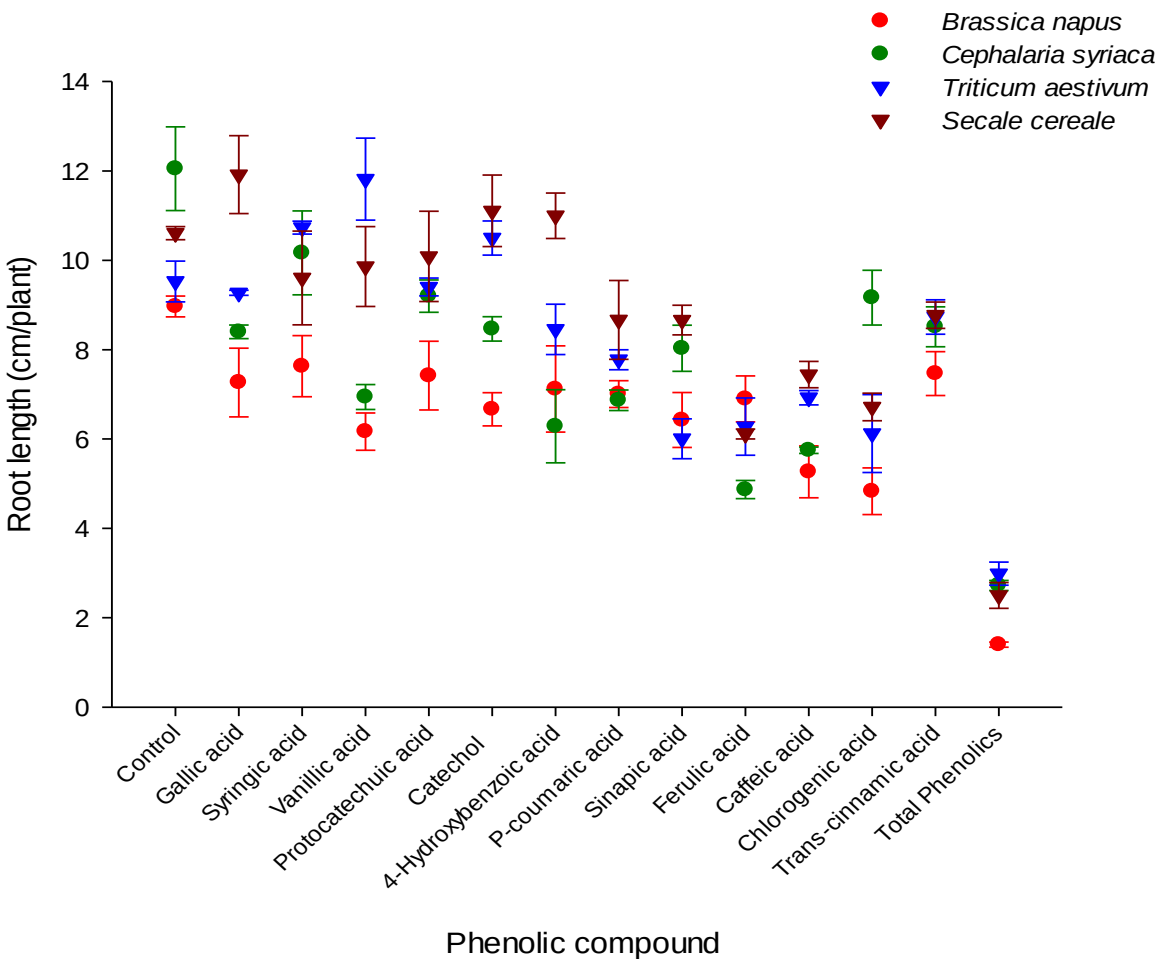


Figure 76 Effects of phenolic compounds on root length of *Brassica napus*, *Cephalaria syriaca*, *Triticum aestivum* and *Secale cereale.*

Concentrations of phenolic compounds are shown in Table 5. Control: distilled water. The results are means of triplicate samples. Error bars represent standard errors. Data analysis was done for all species collectively.

3.4.2.4 Effect of phenolic compounds on shoot dry weight

Effects of phenolic compounds on shoot dry weight of *B. napus*, *C. syriaca*, *T. aestivum* and *S. cereale* are shown in Figure 77. Analysis of variance showed that there were no significant differences between the control and any phenolic acid treatments in the plant species studied.

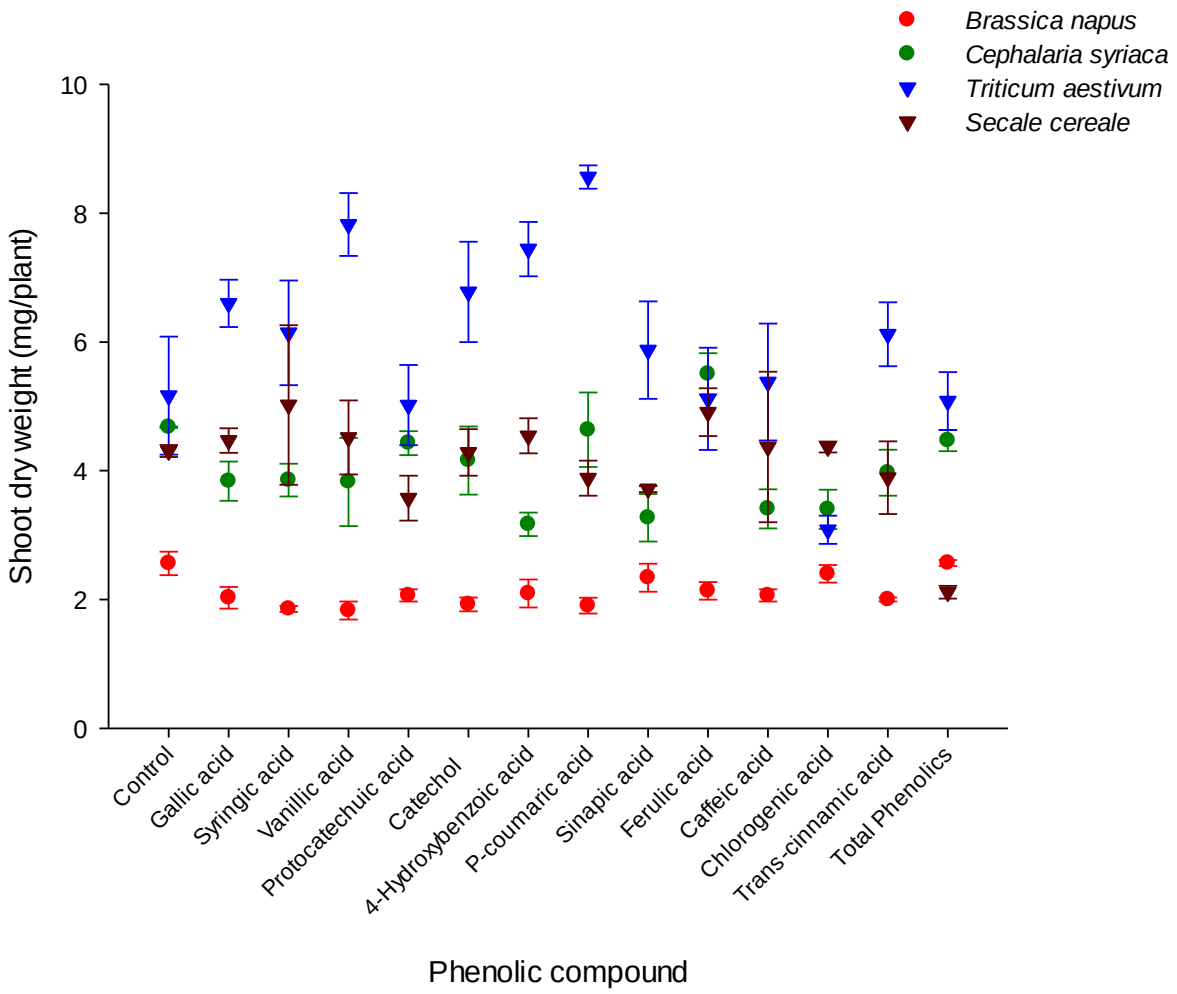


Figure 77 Effects of phenolic compounds on on shoot dry weight of *Brassica napus*, *Cephalaria syriaca*, *Triticum aestivum* and *Secale cereale.*

Concentrations of phenolic compounds are shown in Table 5. Control: distilled water. The results are means of triplicate samples. Error bars represent standard errors. Data analysis was done for all species collectively.

3.4.2.5 Effect of phenolic compounds on root dry weight

Total phenolic compounds caused the greatest reduction of root dry weight of *T. aestivum* and *S. cereale* ($P < 0.001$). Moreover, chlorogenic acid, trans-cinnamic acid and caffeic acid significantly decreased root dry weight of *T. aestivum* (Figure 78). No significant differences were observed between the controls and other phenolic acid treatments.

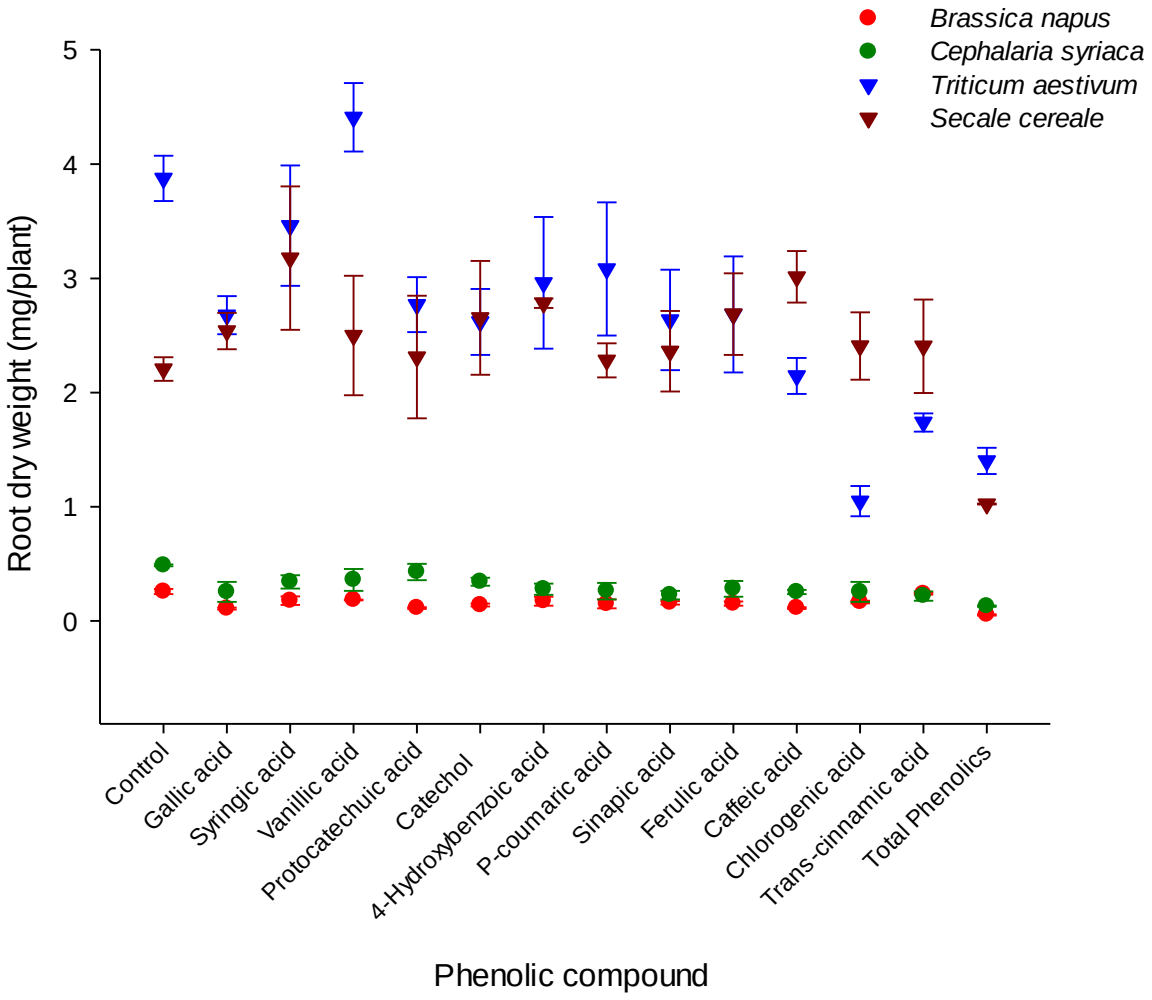


Figure 78 Effect of phenolic compounds on root dry weight of *Brassica napus*, *Cephalaria syriaca*, *Triticum aestivum* and *Secale cereale.*

Concentrations of phenolic compounds are shown in Table 5. Control: distilled water. The results are means of triplicate samples. Error bars represent standard errors. Data analysis was done for all species collectively.

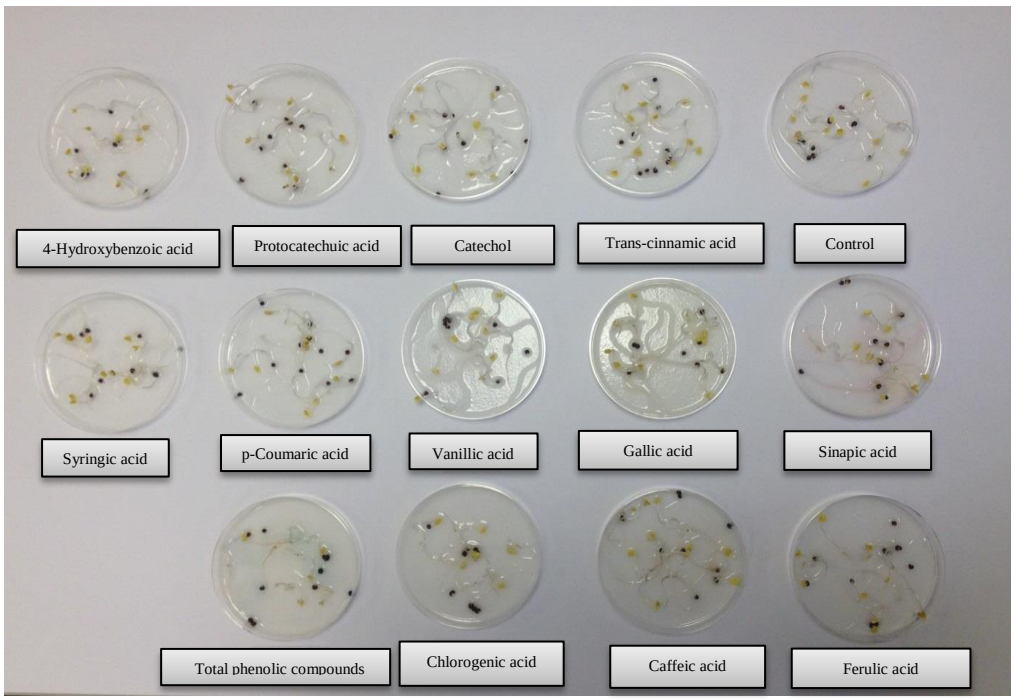


Figure 79 Effect of phenolic compounds on seed germination and seedling growth of *Brassica napus.*

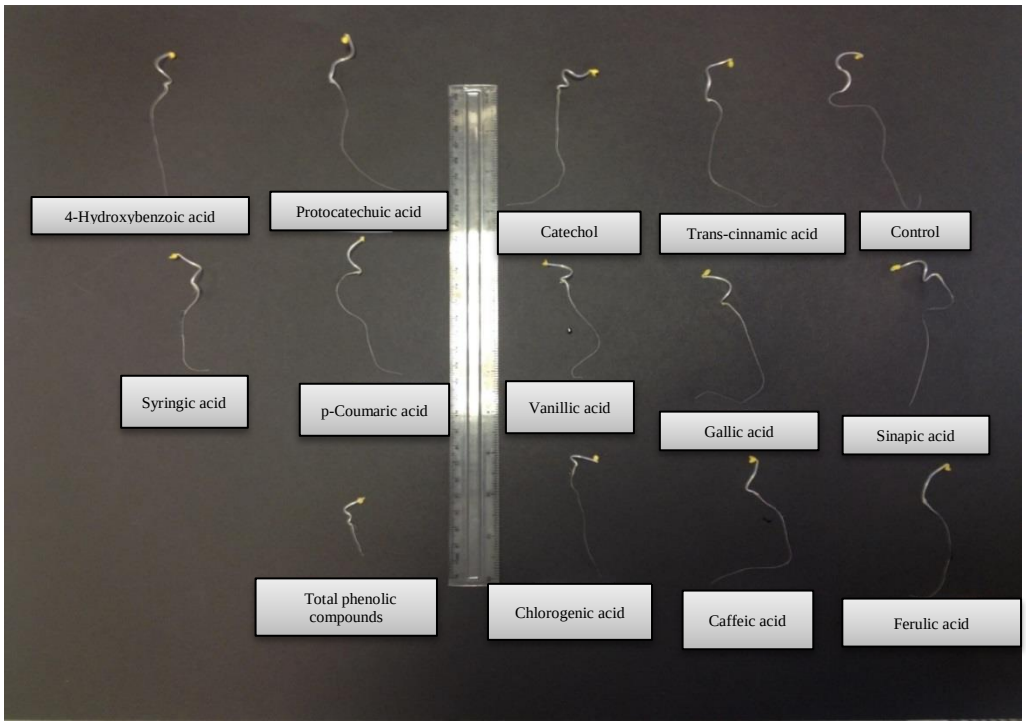


Figure 80 Effects of phenolic compounds on growth of representative seedlings of *Brassica napus.*

Experiment 5

The aims of this experiment were to examine:

- the effects of the herbicide trifluralin at different concentrations on seed germination and growth of *B. napus* and *C. syriaca* in order to determine the most appropriate concentration of trifluralin to use as a positive control with total phenolic compounds and sunflower extracts in further investigations;
- the effects of aqueous shoot extract from two month old sunflower plants, the total phenolic compounds identified within this extract and trifluralin on sugar content, protein content, proline content, DNA content and gibberellic acid (GA) content of *B. napus*, *C. syriaca*, *T. aestivum* and *S. cereale* seedlings;
- the effects of sunflower ground shoot, its identified total phenolic compounds, and trifluralin on seed germination and seedling growth of *S. cereale*, *T. aestivum*, *B. napus* and *C. syriaca* in a pot experiment.

3.5.1 Effect of trifluralin on seed germination and seedling growth of *Brassica napus* and *Cephalaria syriaca*

3.5.1.1 Effect of different concentrations of trifluralin on seed germination of *Brassica napus* and *Cephalaria syriaca*

Seed germination percentages of *B. napus* and *C. syriaca* were significantly ($P < 0.001$) decreased by most concentrations of trifluralin (Figure 81). There was a general trend towards greater reductions in germination at higher concentrations (illustrated in Figures 86, 88).

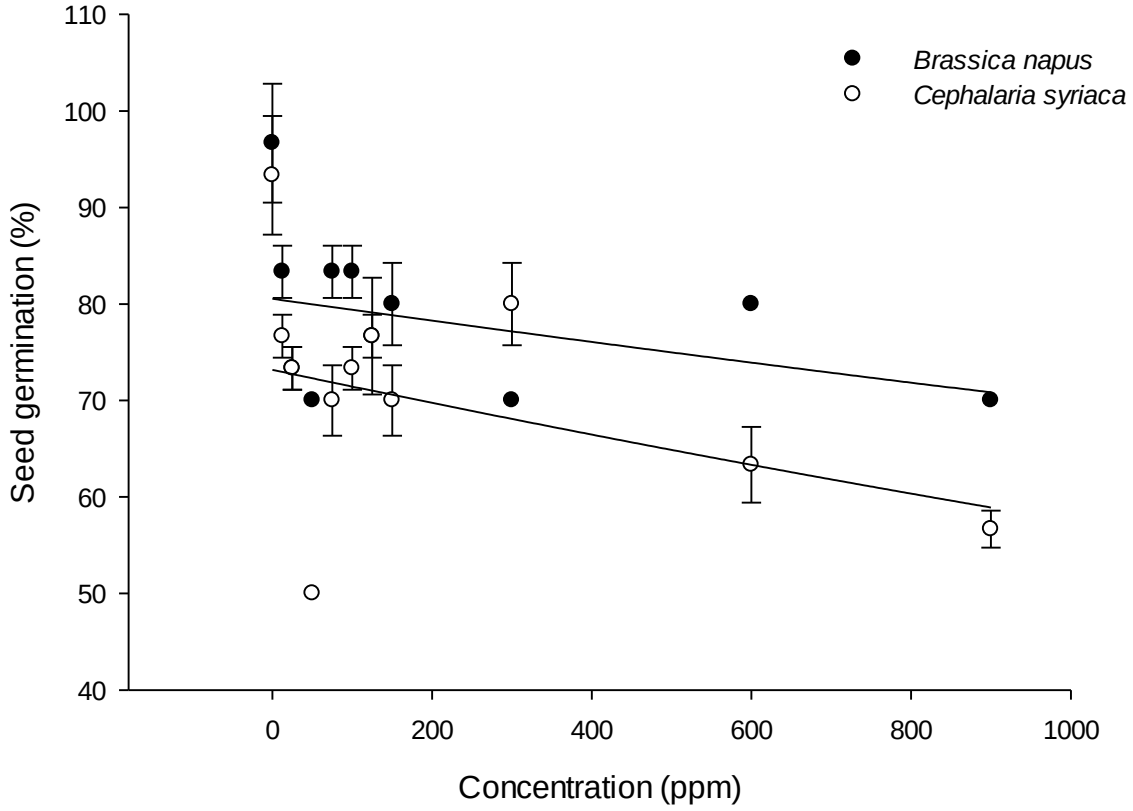


Figure 81 Effects of different concentrations of trifluralin on seed germination of *Brassica napus* and *Cephalaria syriaca.*

The results are means of triplicate samples. Error bars represent standard errors. Data analysis was done for all species collectively. Fitted lines are for exponential decay, calculated using SigmaPlot 12.

3.5.1.2 Effect of trifluralin on shoot length of *Brassica napus* and *Cephalaria syriaca*

Figure 82 shows the effect of different concentrations of trifluralin on shoot length of *B. napus* and *C. syriaca*. Data analysis shows that shoot length was significantly decreased by all trifluralin concentrations. The greatest effect was observed with the highest concentration (900 ppm) in *B. napus* ($P < 0.001$). In contrast the least effect was recorded with the lowest concentration (12.5 ppm).

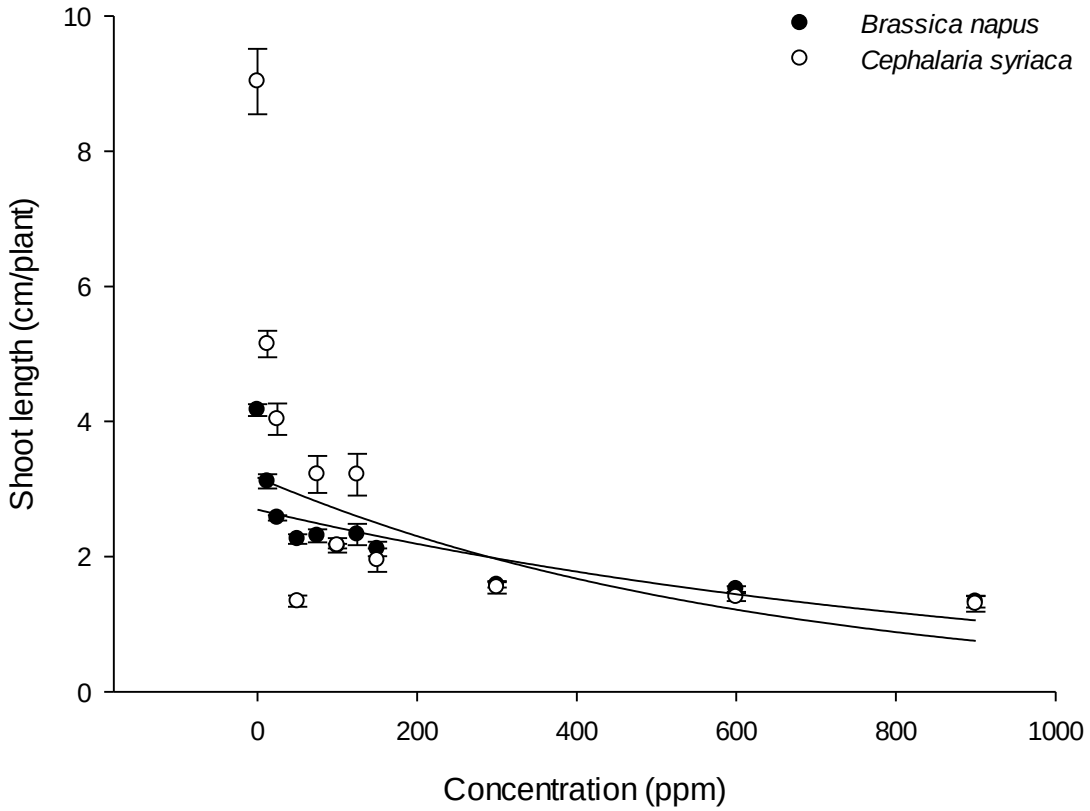


Figure 82 Effect of different concentrations of trifluralin on shoot length of *Brassica napus* and *Cephalaria syriaca.*

The results are means of triplicate samples. Error bars represent standard errors. Data analysis was done for all species collectively. Fitted lines are for exponential decay, calculated using SigmaPlot 12.

3.5.1.3 Effect of trifluralin on root length of *Brassica napus* and *Cephalaria syriaca*

Trifluralin had the greatest effect at the highest concentration (900 ppm) in *B. napus* and *C. syriaca* ($P < 0.001$) (Figure 83). No significant effect was found at a concentration of 12.5 ppm in *B. napus* and at 12.5 and 25 ppm in *C. syriaca* (illustrated in Figure 87).

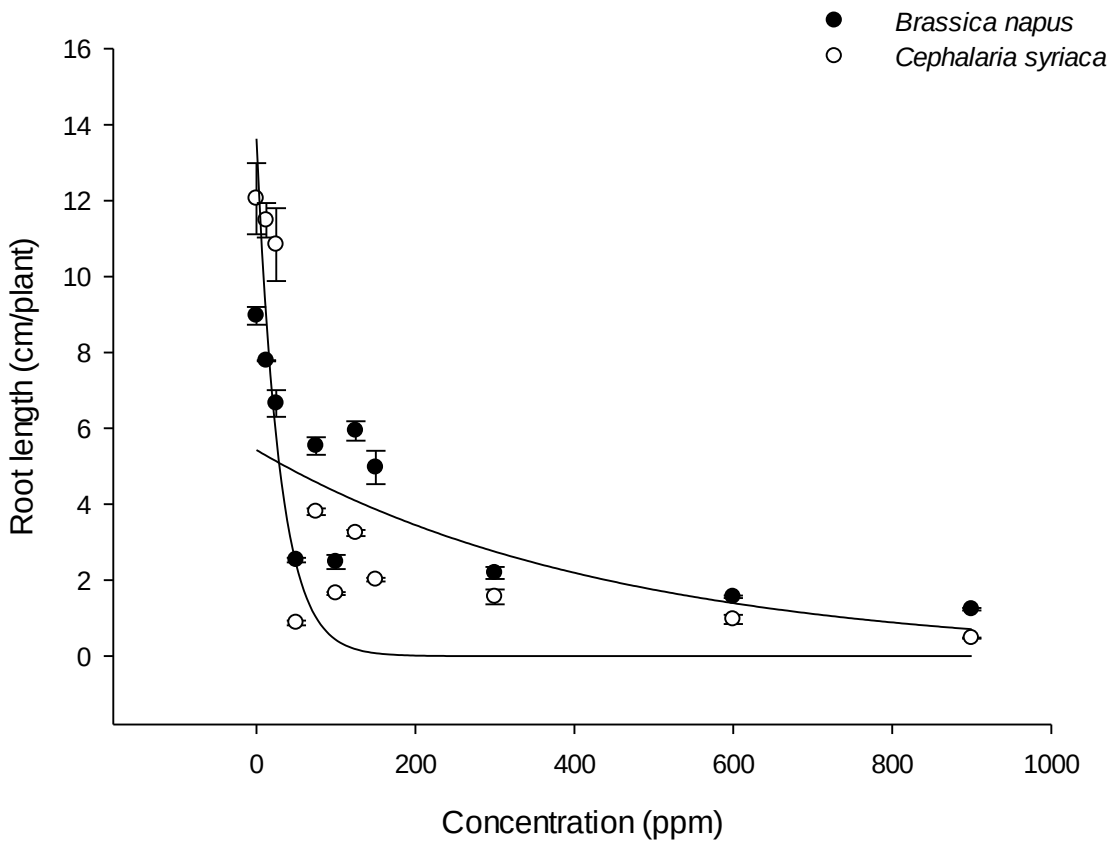


Figure 83 Effect of different concentrations of trifluralin on root length of *Brassica napus* and *Cephalaria syriaca.*

The results are means of triplicate samples. Error bars represent standard errors. Data analysis was done for all species collectively. Fitted lines are for exponential decay, calculated using SigmaPlot 12.

3.5.1.4 Effect of trifluralin on shoot dry weight of *Brassica napus* and *Cephalaria syriaca*

Shoot dry weights of *C. syriaca* were significantly increased ($P < 0.001$) by all concentrations of trifluralin tested compared to the control treatment (Figure 84). However, there was no significant difference between the control treatment and any concentration in *B. napus*.

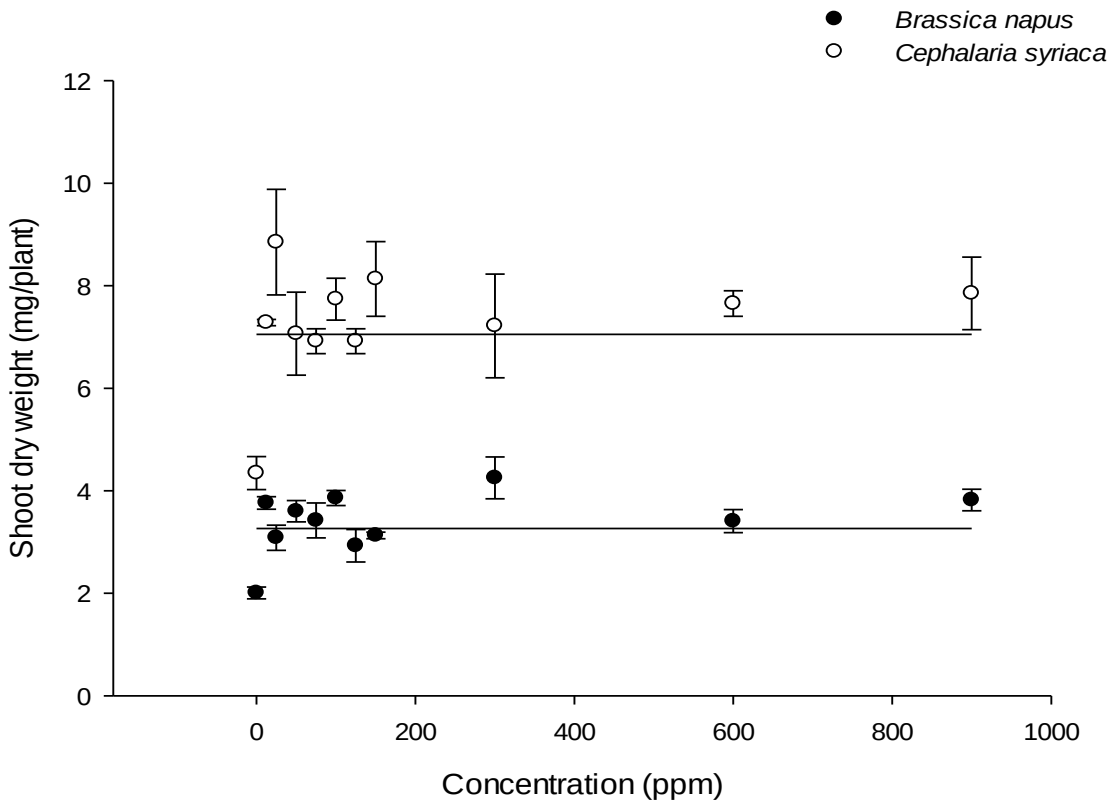


Figure 84 Effect of different concentrations of trifluralin on shoot dry weight of *Brassica napus* and *Cephalaria syriaca.*

The results are means of triplicate samples. Error bars represent standard errors. Data analysis was done for all species collectively. Fitted lines are for exponential decay, calculated using SigmaPlot 12.

3.5.1.5 Effect of trifluralin on root dry weight of *Brassica napus* and *Cephalaria syriaca*

Root dry weight of *C. syriaca* was significantly increased at concentrations of 75, 125 and 150 ppm of trifluralin ($P < 0.001$). There was no significant difference between the control treatment and any trifluralin concentration in *B. napus* (Figure 85).

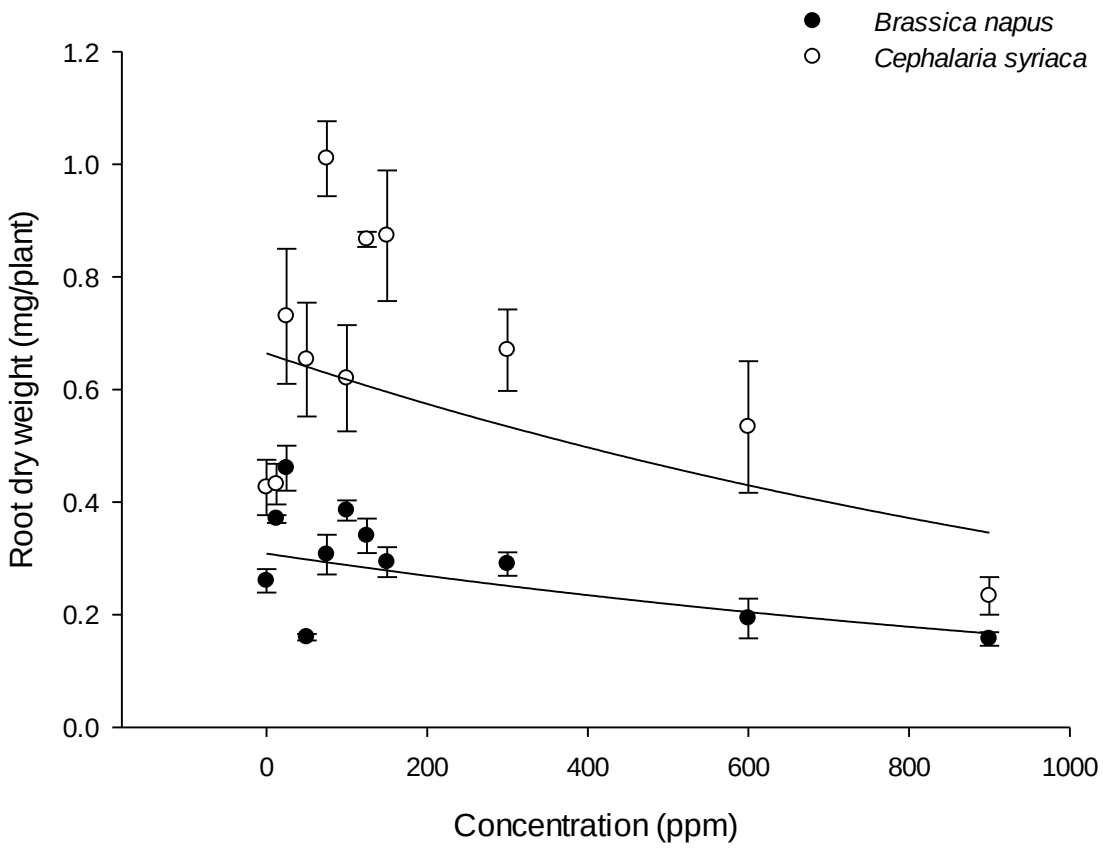


Figure 85 Effect of different concentrations of trifluralin on root dry weight of *Brassica napus* and *Cephalaria syriaca.*

The results are means of triplicate samples. Error bars represent standard errors. Data analysis was done for all species collectively. Fitted lines are for exponential decay, calculated using SigmaPlot 12.

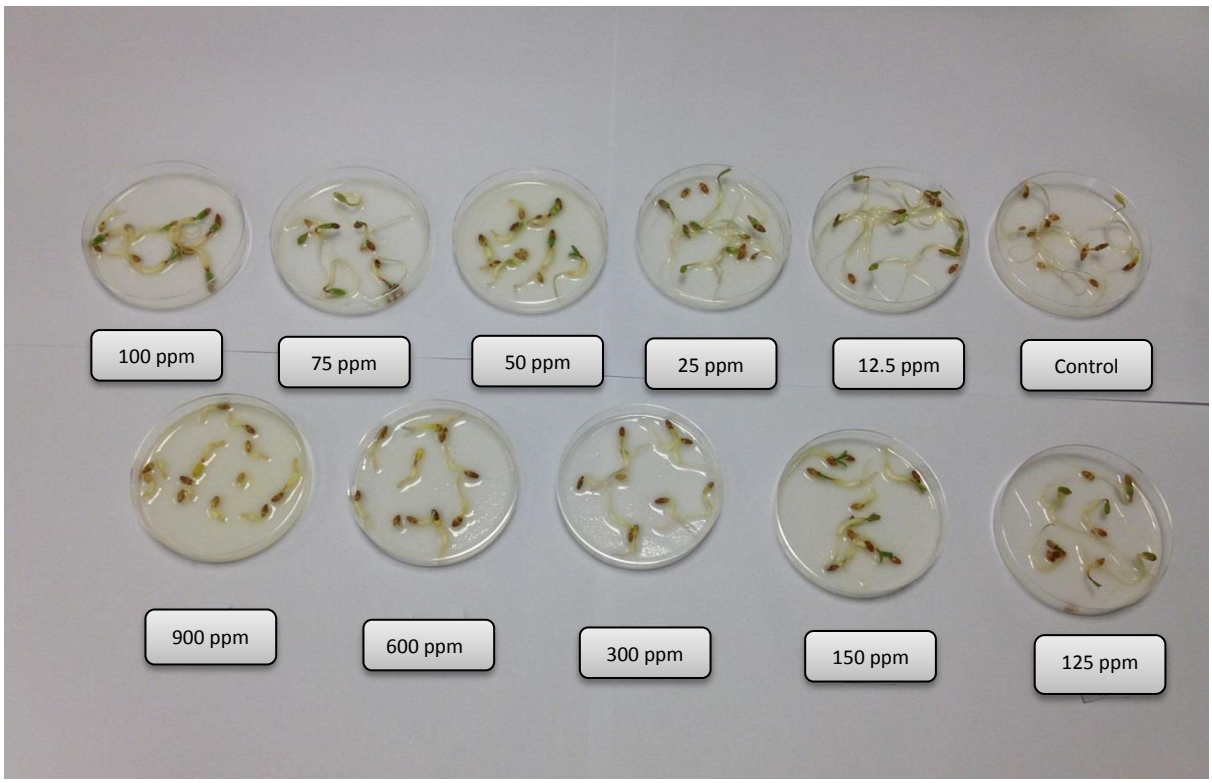


Figure 86 Effect of different concentrations of trifluralin on germination and seedling growth of *Cephalaria syriaca.*

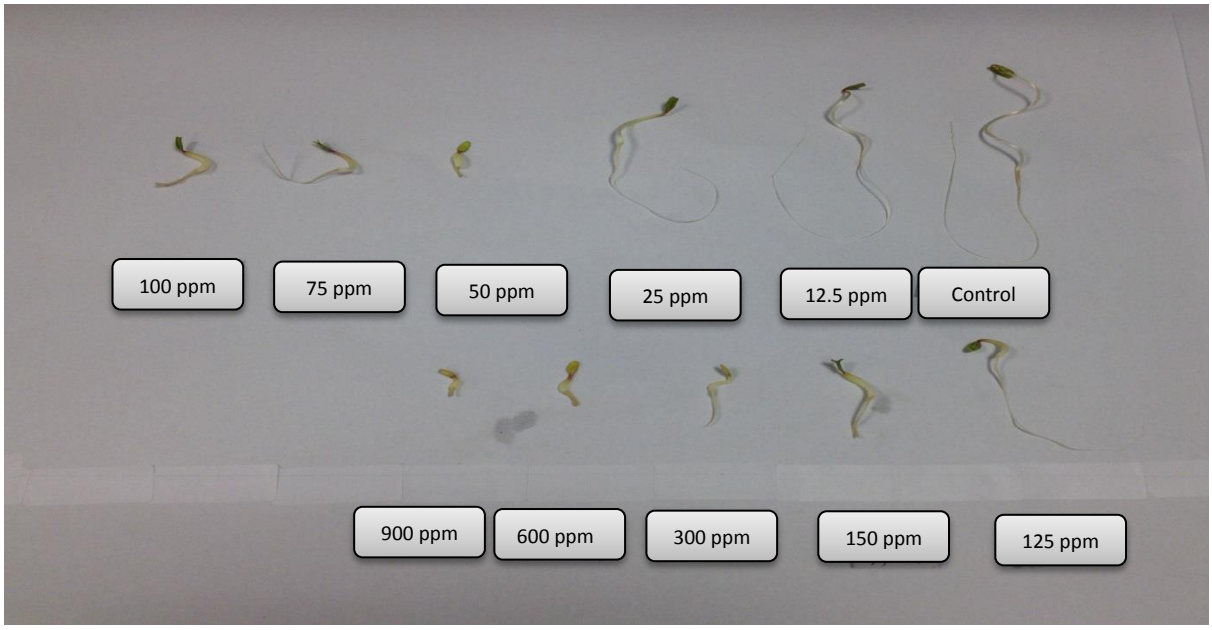


Figure 87 Effects of different concentrations of trifluralin on growth of representative seedlings of *Cephalaria syriaca.*

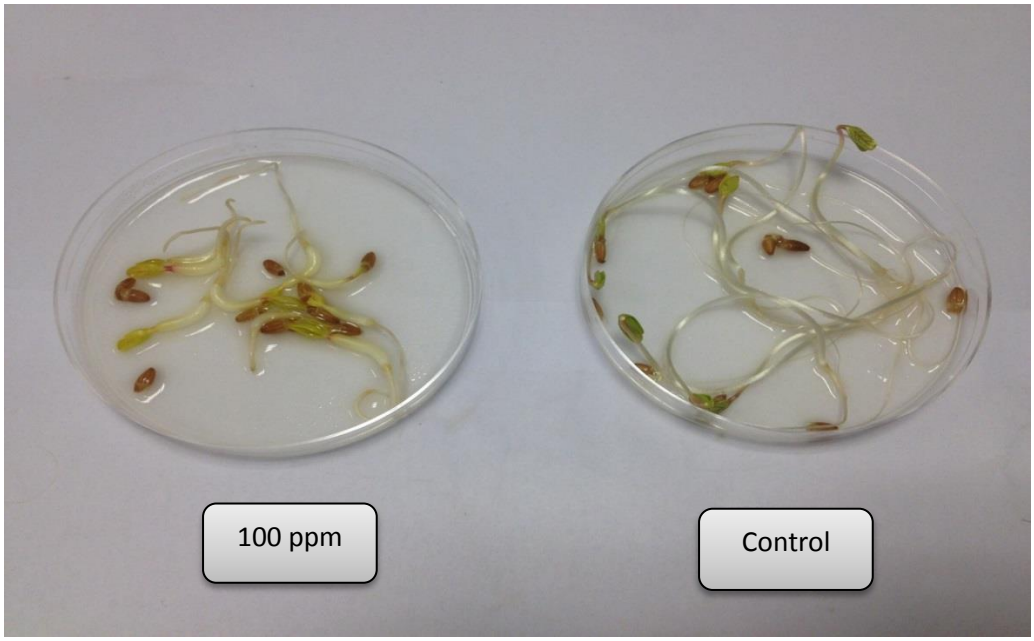


Figure 88 Effect of 100 ppm trifluralin on germination and seedling growth of *Cephalaria syriaca.*

3.5.2 Investigations on the mechanism of effects of aqueous extracts of sunflower shoots, their total phenolic compounds and the herbicide trifluralin on seed germination and early growth of some weed and crop species

3.5.2.1 Effect on sugar content

Figure 89 shows the effects of sunflower shoot aqueous extracts, their identified total phenolic compounds (composition as in Table 5) and trifluralin on sugar content of *S. cereale*, *T. aestivum*, *B. napus*, and *C. syriaca.* Trifluralin caused the greatest reduction in sugar content of all studied species followed by sunflower aqueous shoot extract. However, there was no significant effect of total phenolic compounds on sugar content in *B. napus*.

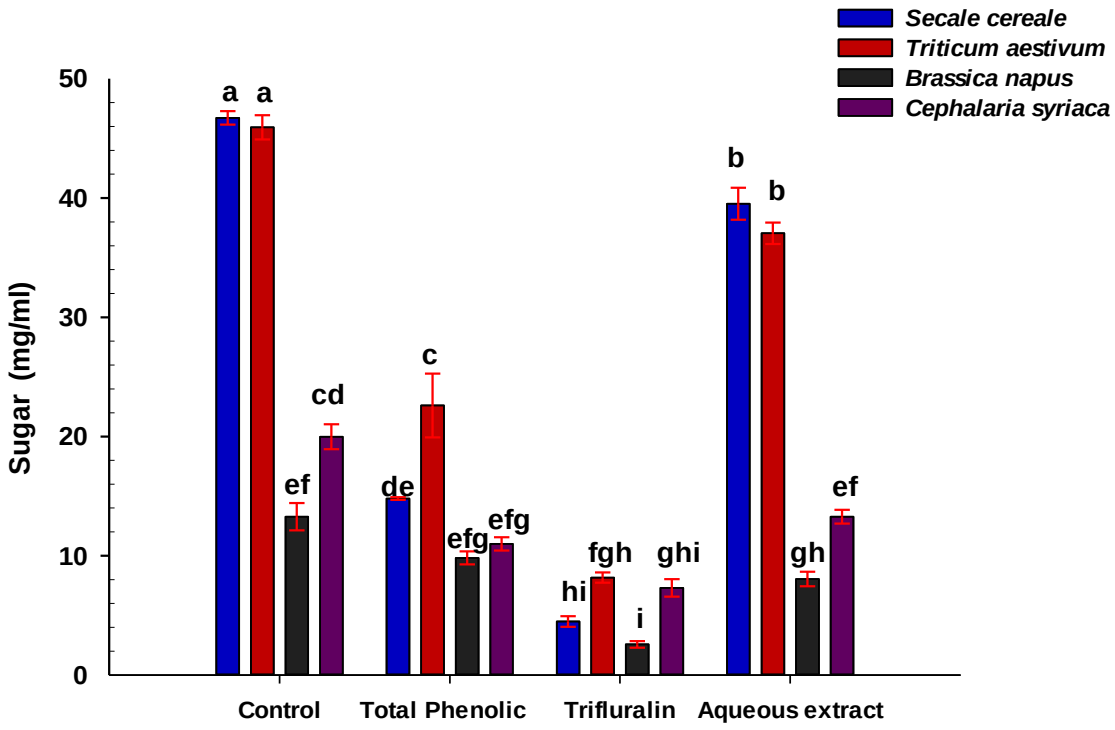


Figure 89 Effect of sunflower shoot aqueous extracts, total sunflower phenolic compounds and trifluralin on sugar content of *Secale cereale*, *Triticum aestivum*, *Brassica napus*, and *Cephalaria syriaca.*

Control: distilled water. Total phenolic compounds: 0.27 mg/ml of distilled water (Table 5), trifluralin: 100 ppm (in distilled water). Two month old sunflower aqueous shoot extract: 3% v/v. Error bars represent standard errors. Significant differences between means were calculated based on Tukey's test ($P \leq 0.05$). Bars with the same letter are not significantly different. The results are means of triplicate samples. Data analysis was done for all species collectively.

3.5.2.2 Effect on protein content

Figure 90 shows that sunflower shoot aqueous extract significantly increased protein in *S. cereale*, *T. aestivum*, and *B. napus.* Trifluralin significantly decreased protein content in *S. cereale* and *T. aestivum.* Total phenolic compounds had the least effect on protein content.

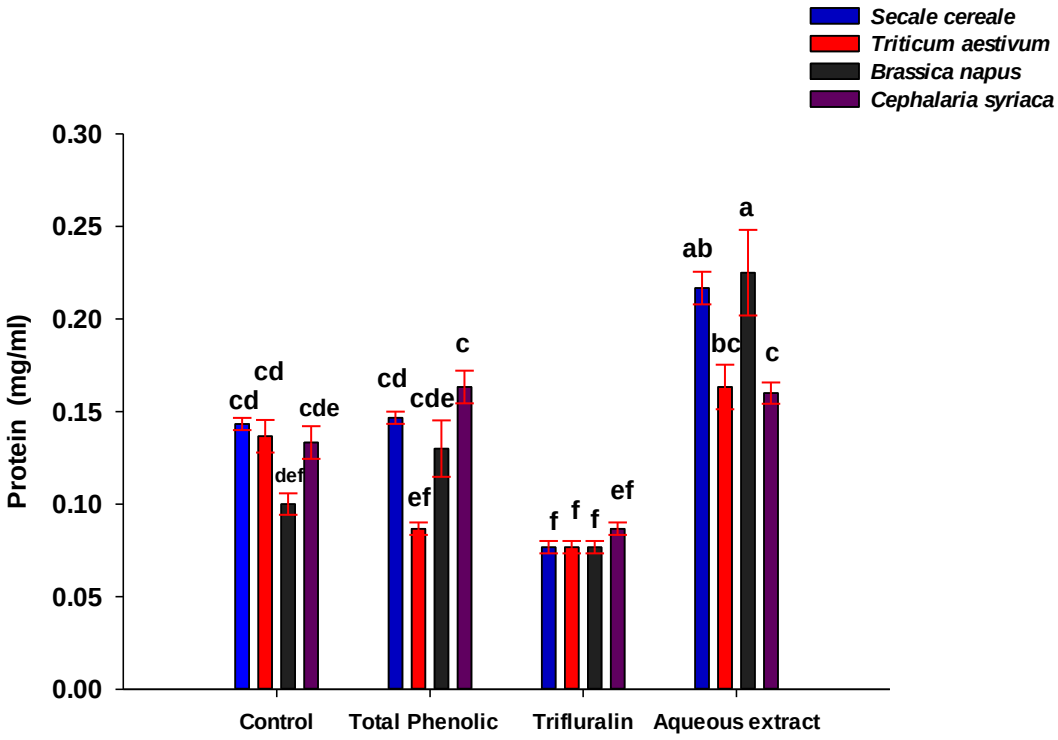


Figure 90 Effect of sunflower shoot aqueous extracts, total sunflower phenolic compounds and trifluralin on protein content of *Secale cereale*, *Triticum aestivum*, *Brassica napus*, and *Cephalaria syriaca.*

Control: distilled water. Total phenolic compounds: 0.27 mg/ml of distilled water (Table 5), trifluralin: 100 ppm (in distilled water). Two month old sunflower aqueous shoot extract: 3% v/v. Error bars represent standard errors. Significant differences between means were calculated based on Tukey's test ($P \leq 0.05$). Bars with the same letter are not significantly different. The results are means of triplicate samples. Data analysis was done for all species collectively.

3.5.2.3 Effect on proline content

Total phenolic compounds significantly reduced proline content in *S. cereale* and *C. syriaca*, while trifluralin only caused a significant reduction in proline in *S. cereale*. No significant difference was recorded between sunflower aqueous shoot extract and the controls (Figure 91).

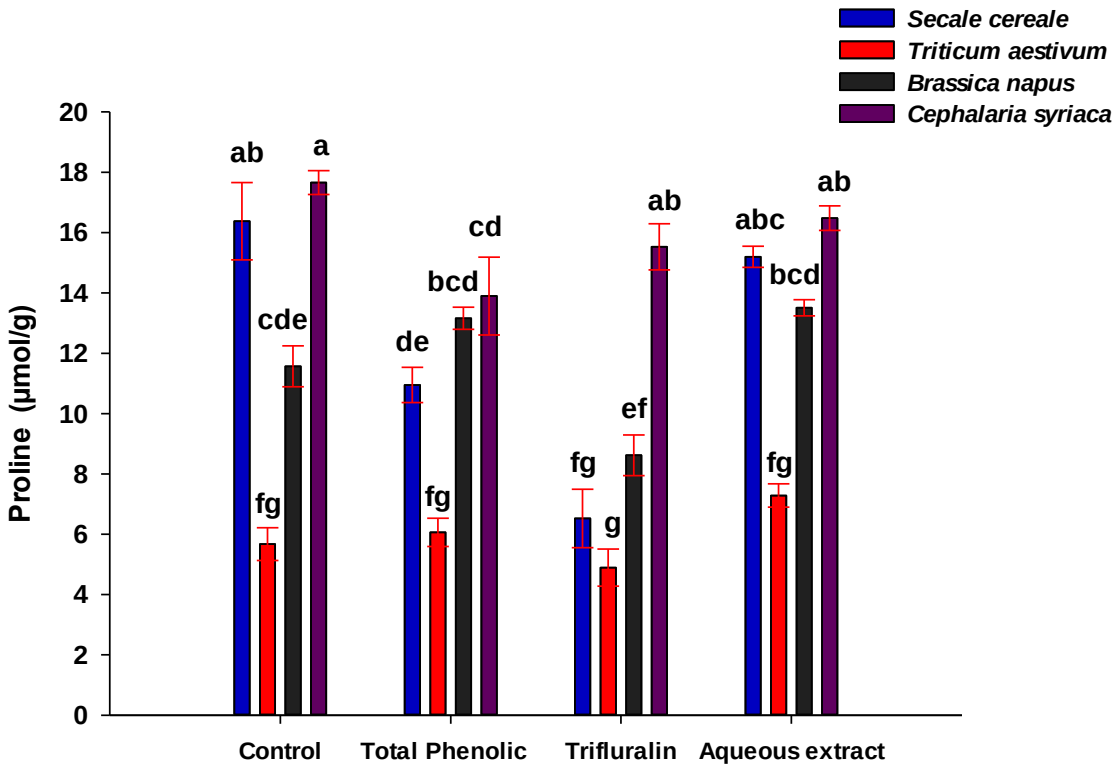


Figure 91 Effect of sunflower shoot aqueous extracts, total sunflower phenolic compounds and trifluralin on proline content of *Secale cereale*, *Triticum aestivum*, *Brassica napus*, and *Cephalaria syriaca.*

Control: distilled water. Total phenolic compounds: 0.27 mg/ml of distilled water (Table 5), trifluralin: 100 ppm (in distilled water). Two month old sunflower aqueous shoot extract: 3% v/v. Error bars represent standard errors. Significant differences between means were calculated based on Tukey's test ($P \leq 0.05$). Bars with the same letter are not significantly different. The results are means of triplicate samples. Data analysis was done for all species collectively.

3.5.2.4 Effect on total DNA content

Figure 92 shows that DNA content of *S. cereale* and *T. aestivum* was significantly reduced by application of sunflower shoot aqueous extract, total phenolic compounds and trifluralin. No significant effect on total DNA was observed in *B. napus* and *C. syriaca.*

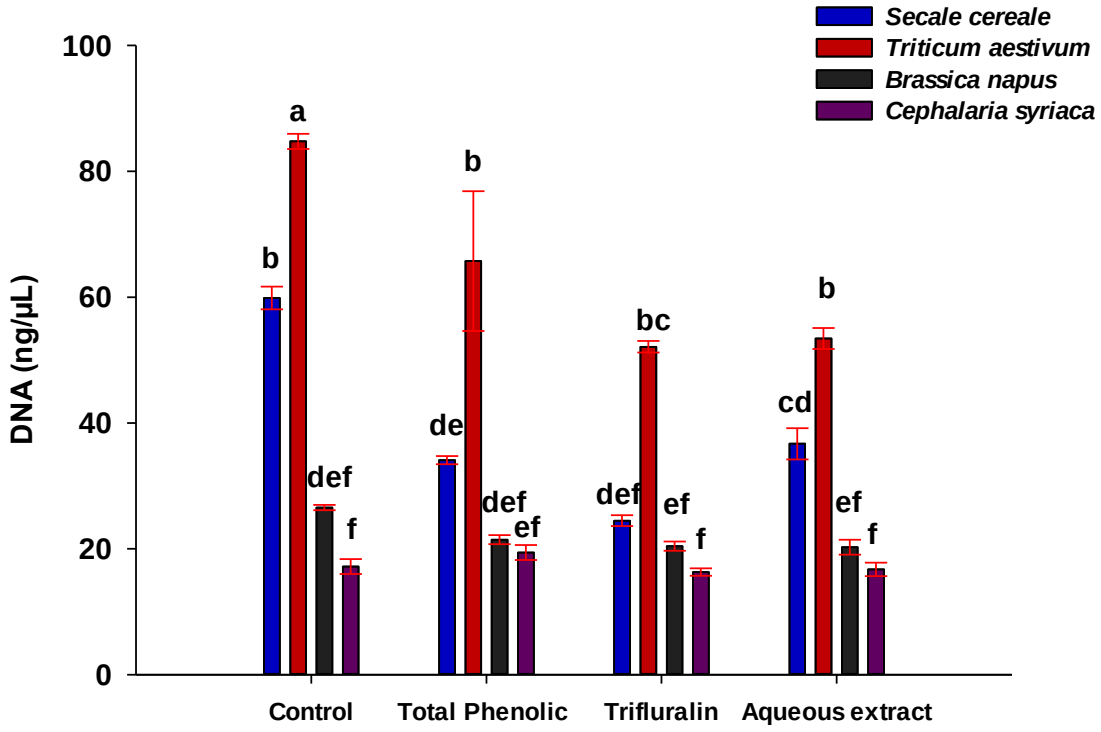


Figure 92 Effect of sunflower shoot aqueous extracts, total sunflower phenolic compounds and trifluralin on total DNA content of *Secale cereale*, *Triticum aestivum*, *Brassica napus*, and *Cephalaria syriaca.*

Control: distilled water. Total phenolic compounds: 0.27 mg/ml of distilled water (Table 5), trifluralin: 100 ppm (in distilled water). Two month old sunflower aqueous shoot extract: 3% v/v. Error bars represent standard errors. Significant differences between means were calculated based on Tukey's test ($P \leq 0.05$). Bars with the same letter are not significantly different. The results are means of triplicate samples. Data analysis was done for all species collectively.

3.5.2.5 Effect on plant hormones

3.5.2.5.1 Effect on gibberellic acid (GA)

The results from testing the effect of sunflower shoot aqueous extracts, their total phenolic compounds and trifluralin on gibberellic acid show that GA concentration in *B. napus* was significantly reduced by the application of all treatments. However, there were no significant differences between treatments and control in *S. cereale*, *T. aestivum* and *C. syriaca* (Figure 93).

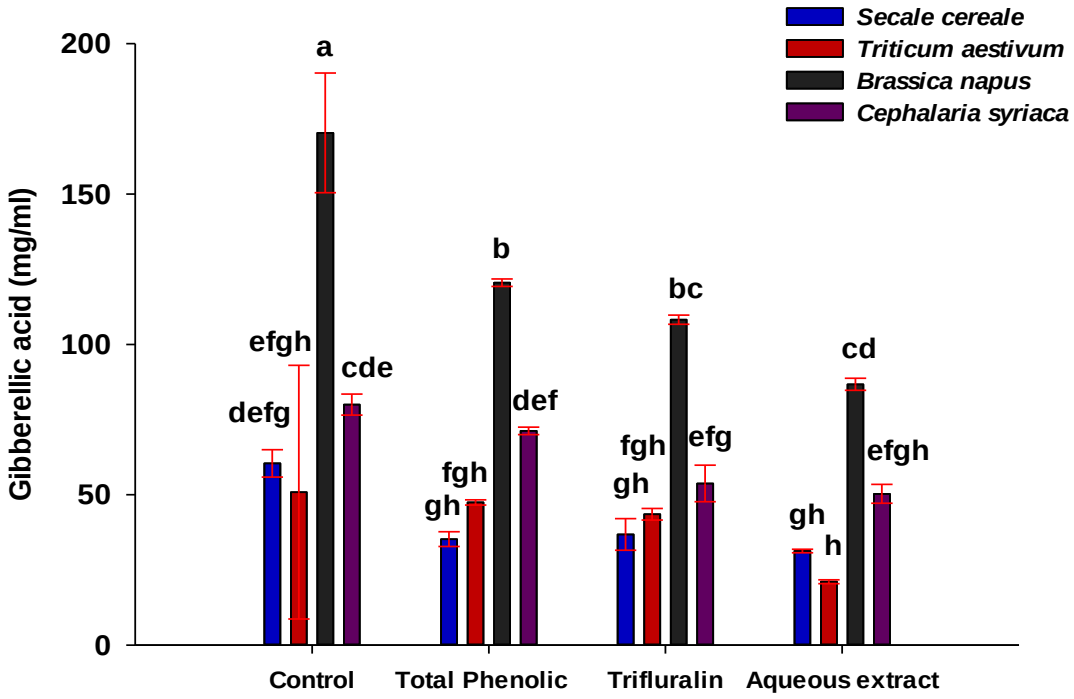


Figure 93 Effect of sunflower shoot aqueous extracts, total sunflower phenolic compounds and trifluralin on gibberellic acid of *Secale cereale*, *Triticum aestivum*, *Brassica napus*, and *Cephalaria syriaca.*

Control: distilled water. Total phenolic compounds: 0.27 mg/ml of distilled water (Table 5), trifluralin: 100 ppm (in distilled water). Two month old sunflower aqueous shoot extract: 3% v/v. Error bars represent standard errors. Significant differences between means were calculated based on Tukey's test ($P \leq 0.05$). Bars with the same letter are not significantly different. The results are means of triplicate samples. Data analysis was done for all species collectively.

3.5.2.5.2 Effect on abscisic acid (ABA)

ABA in *C. syriaca* was significantly increased by the application of sunflower shoot aqueous extracts and reduced by their total phenolic compounds and trifluralin. Total phenolic compounds and sunflower shoot extracts significantly reduced ABA in *S. cereale* and *C. syriaca* (Figure 94). However, no ABA was detected in *T. aestivum*.

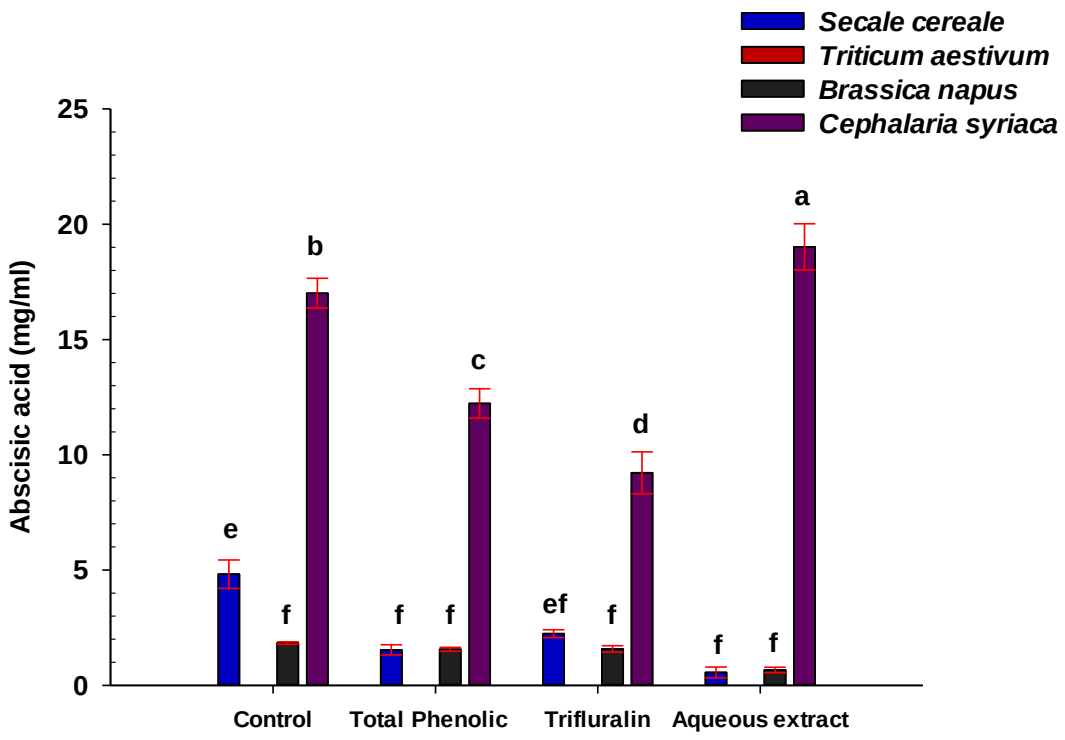


Figure 94 Effect of sunflower shoot aqueous extracts, total sunflower phenolic compounds and trifluralin on abscisic acid of *Secale cereale*, *Triticum aestivum*, *Brassica napus*, and *Cephalaria syriaca.*

Control: distilled water. Total phenolic compounds: 0.27 mg/ml of distilled water (Table 5), trifluralin: 100 ppm (in distilled water). Two month old sunflower aqueous shoot extract: 3% v/v. Error bars represent standard errors. Significant differences between means were calculated based on Tukey's test ($P \leq 0.05$). Bars with the same letter are not significantly different. The results are means of triplicate samples. Data analysis was done for all species collectively.

3.5.2.5.3 Effect on indole acetic acid (IAA)

Figure 95 shows that sunflower shoot aqueous extract and total phenolic compounds significantly reduced indole acetic acid content in all studied plant species (*S. cereale, T. aestivum, B. napus*, and *C. syriaca*). However, trifluralin only caused a significant reduction in *T. aestivum, B. napus* and *C. syriaca.*

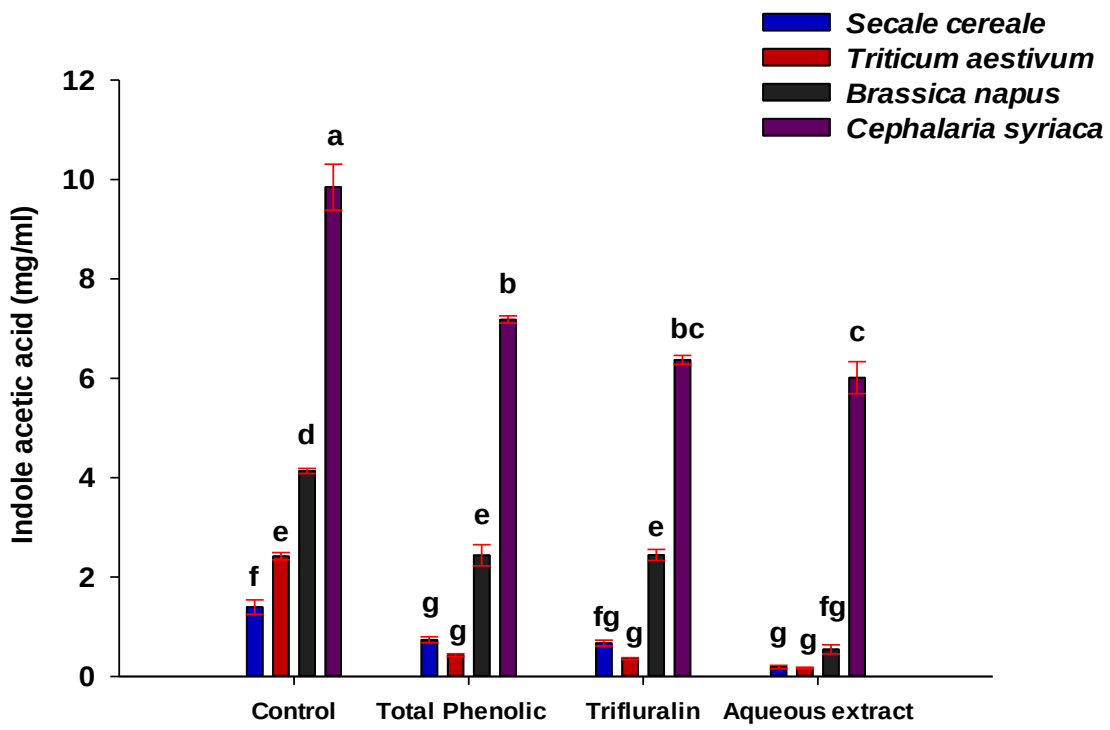


Figure 95 Effect of sunflower shoot aqueous extracts, total sunflower phenolic compounds and trifluralin on indole acetic acid of *Secale cereale*, *Triticum aestivum*, *Brassica napus*, and *Cephalaria syriaca.*

Control: distilled water. Total phenolic compounds: 0.27 mg/ml of distilled water (Table 5), trifluralin: 100 ppm (in distilled water). Two month old sunflower aqueous shoot extract: 3% v/v. Error bars represent standard errors. Significant differences between means were calculated based on Tukey's test ($P \leq 0.05$). Bars with the same letter are not significantly different. The results are means of triplicate samples. Data analysis was done for all species collectively.

3.5.3 Effect of sunflower ground shoot, its identified total phenolic compounds and trifluralin on seed germination and growth of *Secale cereale*, *Triticum aestivum*, *Brassica napus*, and *Cephalaria syriaca* in pot experiment

3.5.3.1 Effect on seed germination

Figure 96 shows the effect of sunflower ground shoot, total phenolic compounds, and trifluralin on seed germination of *S. cereale*, *T. aestivum*, *B. napus*, and *C. syriaca.* Trifluralin caused significant inhibition of seed germination of all plant species compared to the control treatment while total phenolic acid had no significant effect on seed germination. Seed germination of *S. cereale*, *B. napus* and *C. syriaca* was significantly inhibited by the application of sunflower ground shoot (illustrated in Figure 101).

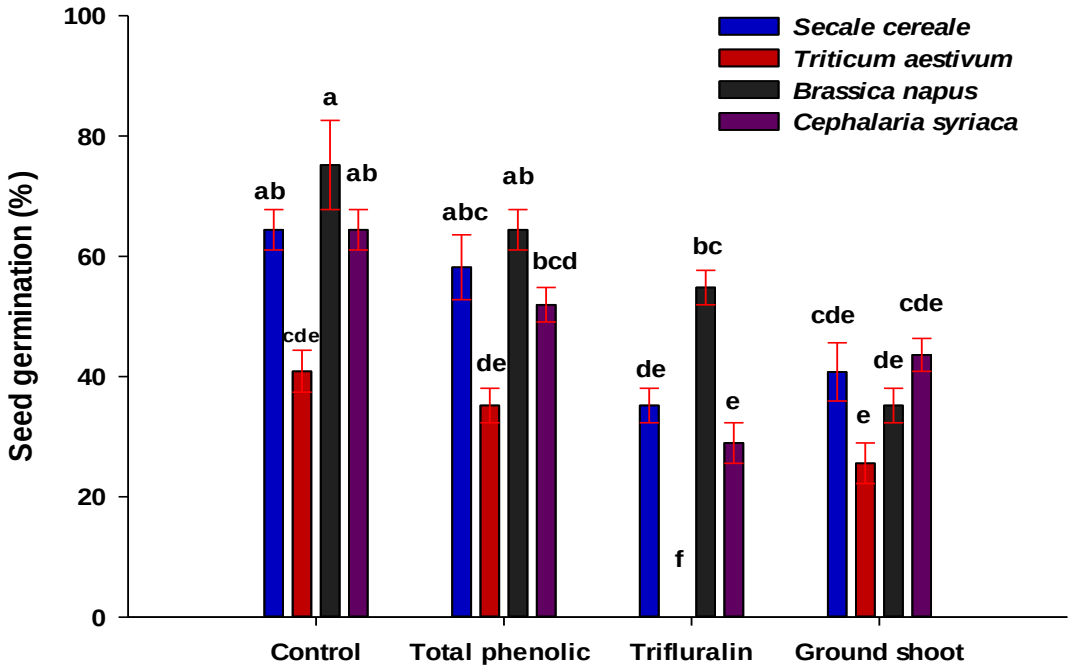


Figure 96 Effect of sunflower ground shoot, total sunflower phenolic compounds and trifluralin on seed germination of *Secale cereale*, *Triticum aestivum*, *Brassica napus*, and *Cephalaria syriaca.*

Control: distilled water. Total phenolic compounds: 0.27 mg/ml of distilled water (Table 5), trifluralin: 100 ppm (in distilled water). Two month old sunflower aqueous shoot extract: 3% v/v. Error bars represent standard errors. Significant differences between means were calculated based on Tukey's test ($P \leq 0.05$). Bars with the same letter are not significantly different. The results are means of triplicate samples. Data analysis was done for all species collectively.

3.5.3.2 Effect on shoot length

Trifluralin dramatically reduced shoot length of all plant species studied in the experiment. Total phenolic compounds only reduced shoot length of *T. aestivum* and *B. napus.* Sunflower ground shoot significantly reduced shoot length of *T. aestivum*, *B. napus*, and *C. syriaca* (Figure 97).

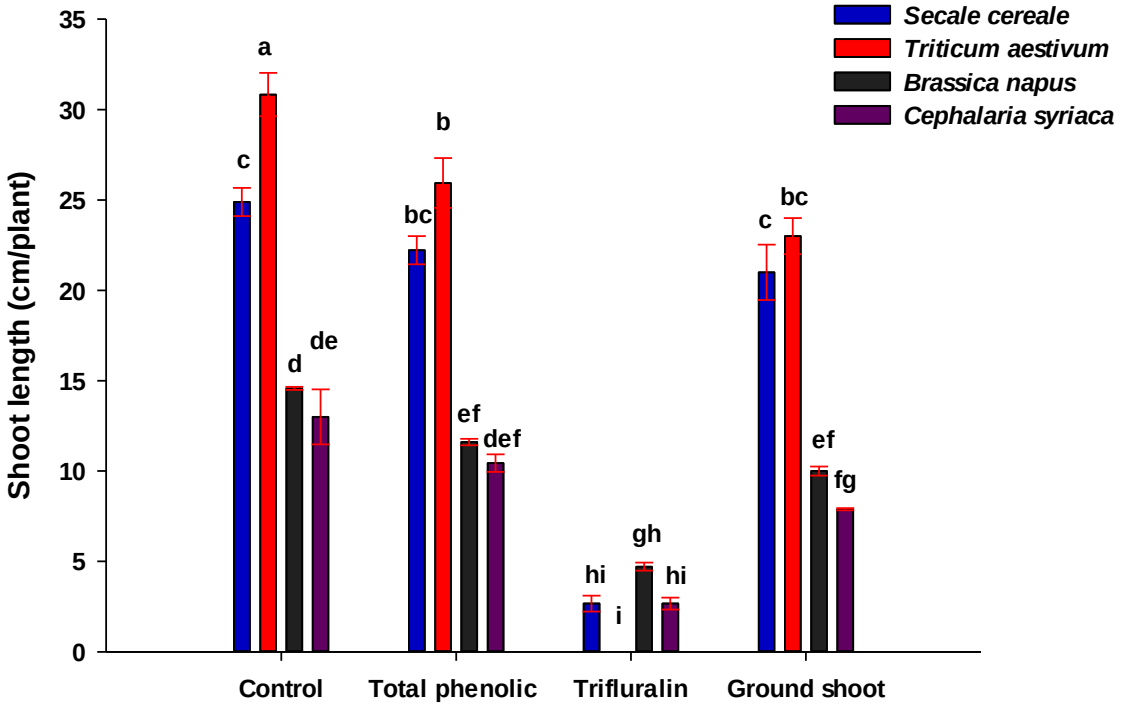


Figure 97 Effect of sunflower ground shoot, total sunflower phenolic compounds and trifluralin on shoot length of *Secale cereale*, *Triticum aestivum*, *Brassica napus*, and *Cephalaria syriaca.*

Control: distilled water. Total phenolic compounds: 0.27 mg/ml of distilled water (Table 5), trifluralin: 100 ppm (in distilled water). Two month old sunflower aqueous shoot extract: 3% v/v. Error bars represent standard errors. Significant differences between means were calculated based on Tukey's test ($P \leq 0.05$). Bars with the same letter are not significantly different. The results are means of triplicate samples. Data analysis was done for all species collectively.

3.5.3.3 Effect on root length

Figure 98 shows that the most significant reduction of root length was caused by trifluralin, which significantly reduced root length of *Secale cereale*, *T. aestivum*, and *C. syriaca* (illustrated in Figures 101, 102). Total phenolic compounds had a significant effect on root length of *S. cereale* and *C. syriaca* while sunflower ground shoot significantly reduced root length of *S. cereale*, *T. aestivum* and *C. syriaca.*

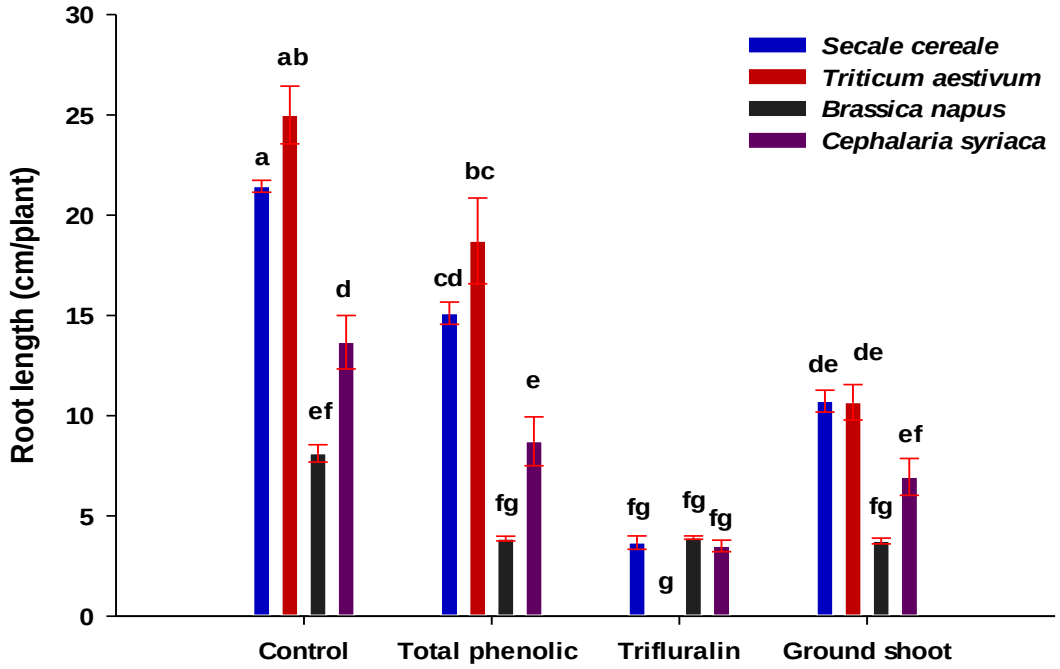


Figure 98 Effect of sunflower ground shoot, total sunflower phenolic compounds and trifluralin on root length of *Secale cereale*, *Triticum aestivum*, *Brassica napus*, and *Cephalaria syriaca.*

Control: distilled water. Total phenolic compounds: 0.27 mg/ml of distilled water (Table 5), trifluralin: 100 ppm (in distilled water). Two month old sunflower aqueous shoot extract: 3% v/v. Error bars represent standard errors. Significant differences between means were calculated based on Tukey's test ($P \leq 0.05$). Bars with the same letter are not significantly different. The results are means of triplicate samples. Data analysis was done for all species collectively.

3.5.3.4 Effect on shoot dry weight

Shoot dry weight was significantly affected by trifluralin in all studied plant species (*S. cereale*, *T. aestivum*, *B. napus*, and *C. syriaca*.). Other treatments were less effective (Figure 99). Total phenolic compounds and sunflower ground shoot only significantly reduced shoot dry weight of *C. syriaca*.

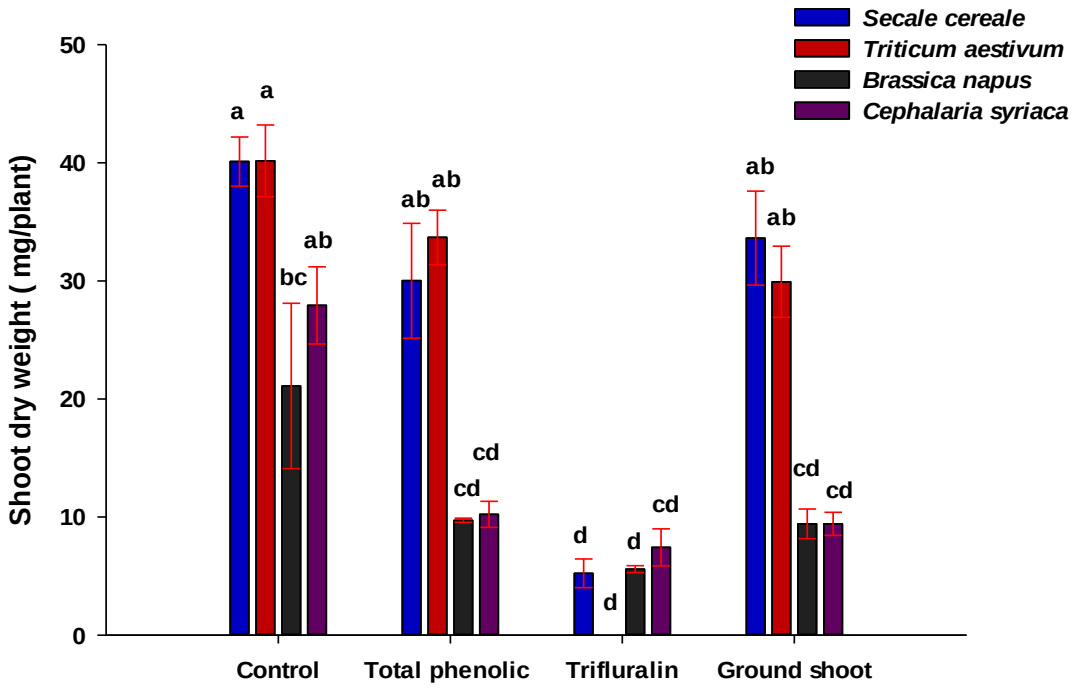


Figure 99 Effect of sunflower ground shoot, total sunflower phenolic compounds and trifluralin on shoot dry weight of *Secale cereale*, *Triticum aestivum*, *Brassica napus*, and *Cephalaria syriaca.*

Control: distilled water. Total phenolic compounds: 0.27 mg/ml of distilled water (Table 5), trifluralin: 100 ppm (in distilled water). Two month old sunflower aqueous shoot extract: 3% v/v. Error bars represent standard errors. Significant differences between means were calculated based on Tukey's test ($P \leq 0.05$). Bars with the same letter are not significantly different. The results are means of triplicate samples. Data analysis was done for all species collectively.

3.5.3.5 Effect on root dry weight

Root dry weights of *S. cereale* and *T. aestivum* were significantly reduced by the application of trifluralin. However, total phenolic compounds and sunflower ground shoot had no significant effect on root dry weight of any plant species (Figure 100).

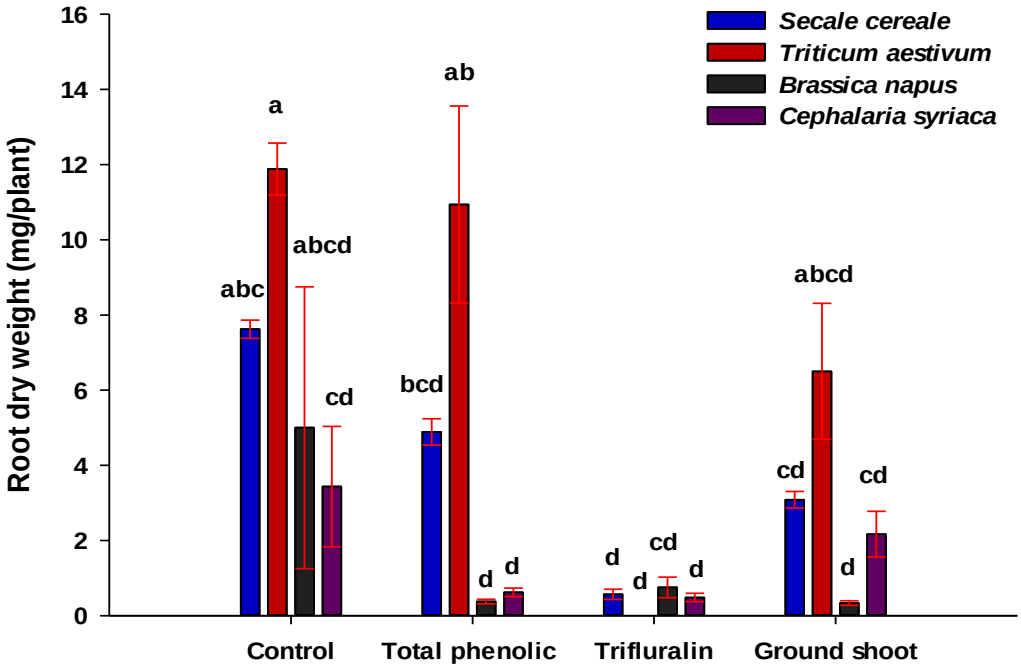


Figure 100 Effect of sunflower ground shoot, total sunflower phenolic compounds and trifluralin on root dry weight of *Secale cereale*, *Triticum aestivum*, *Brassica napus*, and *Cephalaria syriaca*.

Control: distilled water. Total phenolic compounds: 0.27 mg/ml of distilled water (Table 5), trifluralin: 100 ppm (in distilled water). Two month old sunflower aqueous shoot extract: 3% v/v. Error bars represent standard errors. Significant differences between means were calculated based on Tukey's test ($P \leq 0.05$). Bars with the same letter are not significantly different. The results are means of triplicate samples. Data analysis was done for all species collectively.

Figure 101 Effects of sunflower ground shoot, total sunflower phenolic compounds and trifluralin on seed germination and growth of *Cephalaria syriaca.*

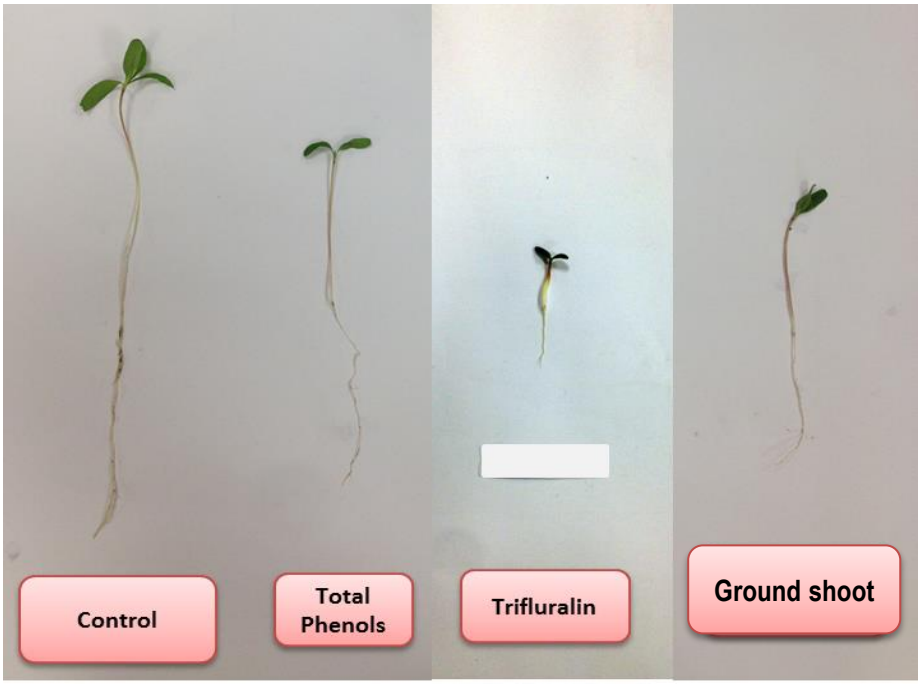


Figure 102 Effect of sunflower ground shoot, total sunflower phenolic compounds and trifluralin on growth of representative seedlings of *Cephalaria syriaca.*

CHAPTER 4 DISCUSSION

Experiment 1

4.1.1 Effects of aqueous shoot and root extracts at the different growing stages of sunflower plants on germination and early growth of *Brassica napus, Secale cereale, Cephalaria syriaca, Phalaris minor, Pisum sativum, Triticum aestivum, Avena fatua* and *Helianthus annuus*.

The allelopathic potential of sunflower shoot and root extracts on some crop and weed species was investigated. In general, the findings show that two month old sunflower shoot extract gave the greatest reduction in seed germination and early growth. Furthermore, sunflower aqueous shoot extract had a greater effect than sunflower aqueous root extract.

There are numerous studies that report that sunflower shoot aqueous extracts have negative impacts on seed germination (Bernat *et al.*, 2004b; Bogatek *et al.*, 2006; Bradosti, 2007; Kamal, 2010; Nikneshan *et al.*, 2011b; Rawat *et al.*, 2012b). Previous studies showed that extracts from different plant parts may differ in their allelopathic effects on seed germination and growth of other plants (Kamal, 2010; Elisante *et al.*, 2013; Kaya *et al.*, 2013). Results of the current study regarding the inhibition of seed germination and seedling growth are similar to previous studies that found that sunflower extracts have negative influence on seed germination of other plants (Irons and Burnside, 1982; Batish *et al.*, 2002; Kamal and Bano, 2008; Alsaadawi *et al.*, 2012).

The reduction of seed germination, shoot and root length, and shoot and root dry weight of the studied plant species may be due to the effect of allelochemicals present in sunflower extracts which could have negative impacts on cell division and physiological activities. Also during the germination process, allelochemicals may cause changes in cell membrane permeability of the studied plant and weed species (Rizvi and Rizvi, 1992). They may change respiration and reduce the concentration of ATP and RNA or disturb the functions of secondary messengers which are necessary for germination and growth (Gatti *et al.*, 2010). Phenolic compounds contribute to the plant growth system and could affect seedling growth of plants through affecting plant hormones (Callaway and Aschehoug, 2000). Muscolo *et al.* (2001) studied the effect of phenolic compounds on respiratory enzymes in seed germination of *Pinus laricio.* The

findings showed that the inhibition of seed germination may due to the strong correlation of phenolic compounds which inhibit glycolysis enzyme activities.

The present study has shown that extracts prepared from shoots of plants that were two months old had a greater effect on seed germination and seedling growth than extracts that were prepared from shoots at the one week, one month and mature stages. However, one month root extract had the most negative impact on seed germination and seedling growth of plant species. These results support Yarnia (2013), who worked on the allelopathic effects of sunflower aqueous extracts of vegetative, inflorescence and mature stages on seed germination and early growth of pigweed (*Amaranthus retroflexus*). The results indicated that leaf extract at vegetative growth stage caused the greatest reduction in seed germination and early growth of pigweed. The results are also in agreement with the results of Movaghatiana and Khorsandib (2014), who showed that wheat aqueous extract at flowering stage caused greater reduction than extracts at mature stage on seed germination and growth of wild mustard. Nevertheless, these findings are not similar to results of Ali (2009), who studied the allelopathic potential of *C. syriaca* at different growth stages (0, 20, 40, 60 and 80 days) on seed germination and early growth of seven plant species. The findings showed that plant age made no significant difference to the effects of extracts on seed germination and seedling growth of the plants studied.

The present study has indicated that sunflower shoot extracts have more inhibitory effects on seed germination, shoot and root length and shoot and root dry weight than root extract (Kamal, 2010; Elisante *et al.*, 2013; Kaya *et al.*, 2013). This might be because water soluble allelochemicals have greater inhibitory effect from sunflower shoot extracts than root extracts. These findings are in agreement with Kamal (2010), who reported that sunflower plant parts differ in their allelopathic potential for affecting germination and growth of wheat (*Triticum aestivum*); sunflower leaf extracts reduced germination and growth more than sunflower root extracts. The results are also in accordance with previously reported findings by Asgharipour and Rafiei (2011), who studied the effects of different concentrations of sunflower stem, leaf, and root aqueous extracts on germination and seedling growth of amaranth and purple nutsedge. Their findings indicated that sunflower leaf aqueous extracts caused greater reduction in seed germination and early growth than root aqueous extracts. Moreover, our findings are in agreement with Munir and Tawaha (2002), who reported that for black mustard the most allelopathic effects can be produced by leaf extracts.

4.1.2 Freeze-dried sunflower shoot and root extract at different growth stages

The dry matter yields of sunflower shoot and root extracts at different growth stages (one week, one month, two month and mature stage) were examined. Two month shoot and one week root extracts gave higher yields than other samples (18.9%, 14.2% respectively). The lowest shoot freeze-dried weight was for one month aqueous extract (11.28%). Furthermore, the lowest root freeze-dried weight was for mature stage root extract (4.31%). Sunflower aqueous shoot extracts at two months growth stage contained the highest concentration of phenolic compounds.

4.1.3 Qualitative chemical test of sunflower shoot extract

Phytochemical screening of sunflower shoot and root extracts at different growth stages (one week, one month, two month and mature stage) was carried out. Tannins, terpenoids, saponins, phenolics and flavonoids were present in both shoot and root extracts at most growth stages, while phlobatannins were only present in root extracts at different growth stages, except at one week. However, alkaloids were not present in sunflower shoot or root extracts. These findings about presence of phenolics and flavonoids are in agreement with a previous study by Kamal (2013).

Experiment 2

4.2.1 Effects of different concentrations of sunflower shoot extract made from plants at the mature stage on germination and early growth of rapeseed (*Brassica napus*), rye (*Secale cereale*), Syrian Cephalaria (*Cephalaria syriaca*) and littleseed canary grass (*Phalaris minor*)

The bioherbicidal effects of sunflower shoot extract (mature stage) at different concentrations on germination and early growth of *B. napus*, *S. cereale*, *C. syriaca* and *P. minor* were studied. The reduction of seed germination and growth, shoot and root length, and shoot and root dry weight of studied plant species may be due to the impact of allelochemicals present in sunflower shoot aqueous extracts (Ghafar *et al.*, 2001; Macías *et al.*, 2002b; Alsaadawi *et al.*, 2012).

The findings about the allelopathic activity of different concentrations of mature stage sunflower shoot extract on germination and early growth of *B. napus*, *S. cereale*, *C. syriaca* and *P. minor* showed that the negative impact increased with increasing concentration of the extract. 5% and 10% aqueous shoot extracts had the most allelopathic influence on seed germination and early growth of the plants. These results are similar to the results obtained by Sharma and Satsangi (2013), who showed that higher concentrations (50-100%) of sunflower shoot aqueous extracts had greater effects than extracts with low concentrations on *Amaranthus viridis* and *Parthenium hysterophorus.*

Our findings indicate that the bioherbicidal effect increased with increasing concentration of sunflower shoot extract. These results are in agreement with previous studies which reported that allelopathic activity increases with increase in concentrations (Rice, 1984; Chon *et al.*, 2003; Peng *et al.*, 2004; Khanh *et al.*, 2005; Gatti *et al.*, 2010; Kamal, 2010). These results are in accordance with a study by Khaliq *et al.* (2012), who investigated the effects of sunflower aqueous extracts at different concentrations (0, 25, 50, 75, and 100%) on seed germination and seedling growth of dragon spurge (*Euphorbia dracunculoides* Lam.). Their findings indicated that sunflower aqueous extract at higher concentrations caused the greatest reduction in seed germination and shoot and root dry weight of *E. dracunculoides.* This also confirms a previous study by Bogatek *et al.* (2006), who reported that increasing concentrations of sunflower leaf extract increased its influence on seed germination of mustard (*Sinapis alba*). The highest concentration (10%) gave the greatest reduction in seed germination of mustard.

However, higher concentrations of extracts may not only increase quantity of allelochemicals but might increase osmotic potential and so affect germination and seedling growth (Kamal, 2010). Einhellig (1995) reported that higher concentrations of extracts contain higher amount of allelochemicals, which give the opportunity for extracts to provide better inhibitory effects. In addition, low concentrations of sunflower shoot and root extracts may not inhibit germination and early growth of plants (Macías *et al.*, 2000). This also confirms a previous study by Ciarka *et al.* (2009) who indicated that sunflower volatiles are promoters at lower concentrations.

The results of the present study indicated that sunflower aqueous shoot extracts significantly decreased shoot and root length and shoot and root dry weight of most studied plant species. These results are in agreement with Ghafar *et al.* (2000).

The inhibition of seed germination, shoot and root length, shoot and root dry weight of the studied plant species may be the result of effect of allelopathic compounds present in sunflower extracts that could probably have inhibitory effect on cell division, respiration and physiological activities (Rizvi and Rizvi, 1992; Gatti *et al.*, 2010).

4.2.2 Total phenolic acid quantification in aqueous shoot extract of sunflower by using spectrophotometer

Sunflower extracts contain phenolic compounds which contribute to allelopathic potential against other plants (Macías *et al.*, 2002a; Kamal, 2010; Alsaadawi *et al.*, 2012). Thus, it is essential to measure total phenolics in sunflower shoot extracts.

Total phenolic concentration (gallic acid equivalent) of freeze-dried sunflower shoot extract (mature stage) was determined. The concentration of total phenolic compounds was 425 µg/ml. These results are broadly in accordance with previous studies on quantification of total phenolic compounds in sunflower extracts (Pedrosa *et al.*, 2000; Ghafar *et al.*, 2001; Nadeem *et al.*, 2011; Kamal, 2013). Pedrosa *et al.* (2000) quantified chlorogenic acid and caffeic acid from sunflower seeds of five different genotypes (Tesoro, Marko, Clip, Vyp-70 and Nanta). They found that chlorogenic acid concentrations of Tesoro, Marko, Clip, Vyp-70 and Nanta were 0.0032, 0.00185, 0.00895, 0.00373, and 0.00882 g/kg respectively and caffeic acid concentrations of these sunflower genotypes were 0.0035, 0.0014, 0.0118, 0.0016, and 0.03110 g/kg. Moreover, Ghafar *et al.* (2001) studied total phenolic compounds of sunflower stems and leaves. They reported that, by assay with Folin-Ciocalteu reagent, concentrations of total phenolic compounds in stems and leaves were 0.016 and 0.0316 mM/g, respectively [units are as in the paper].

4.2.3 Effect of sunflower two month aqueous shoot extract on mitotic index and cell elongation of *Avena fatua* root meristems

The effects of two month sunflower shoot aqueous extract on mitotic index and cell elongation of *Avena fatua* were investigated. The mitotic index value in the control was greater than in the test treatment. However, there was no significant difference between control and test treatments. No significnt different was recorded between mitotic phases (Table 6). On the other hand, the extract significantly reduced cell elongation. In contrast to our results, Jafari *et al.* (2011) reported that rice leaf extract (cultivar Mehr) affects the growth of barnyard grass (*Echinochloa muricata*) through reducing the mitotic index. Secondary metabolites influence phytohormones (gibberellins and auxins) and significantly affect cell elongation in plants (Sharp *et al.*, 2000; Figueiredo *et al.*, 2011; Gamalero and Glick, 2011).

The effect of allelochemicals on cell division might due to disturbance of the cell cycle and damage to cell membranes (Koitabashi *et al.*, 1997; Teerarak *et al.*, 2010; Mohamed and El-Ashry, 2012). Other researchers reported that allelochemicals decrease mitotic index and affect the process of cell division (Dayan *et al.*, 1999; Abrahim *et al.*, 2000; Romagni *et al.*, 2000; Kaur and Kaushik, 2005).

Experiment 3

4.3 Effect of sunflower ground shoot and aqueous extract on germination and early growth of rapeseed (*Brassica napus*), wild oat (*Avena fatua*), Syrian Cephalaria (*Cephalaria syriaca*), and littleseed canary grass (*Phalaris minor*) in pot experiment

4.3.1 Effect of sunflower two month ground shoot and aqueous extract on germination and early growth

Two month sunflower ground shoot (6 and 9 g/pot) significantly inhibited seed germination of most studied plant species, whereas the application of sunflower aqueous shoot extract (3%, 6% and 9%) did not significantly affect seed germination of any studied plant species. Numerous studies indicate that incorporating allelopathic plant ground shoots into the soil can have an inhibitory effect on germination and growth of other plants (Qasem, 1994; Al-Khatib et al., 1997; Alsaadawi et al., 2011). These results are in agreement with Gallandt *et al.* (1999), who mentioned that incorporating allelopathic crop residues into soil has a bioherbicidal influence on weed growth by affecting seed germination and suppressing weed growth. Cheema and Khaliq (2000) showed that the application of sorghum aqueous extract and mixing sorghum stalks with soil suppresses weeds in wheat. It has been reported that sunflower ground shoot has allelopathic effects after incorporation into soil and could be utilized for biological weed management as well as playing an essential role for sustainable agriculture (Khaliq *et al.*, 2011; Rawat *et al.*, 2012a).

The findings confirm that sunflower two month ground shoot had a significantly greater effect on shoot and root length than sunflower two month shoot aqueous extract. Sunflower two month shoot aqueous extract increased shoot length and shoot dry weight of most studied plant species. These findings are in accordance with previous studies, which reported that low concentrations of sunflower shoot and root extract may not inhibit germination and early growth of plants (Macías *et al.*, 2000). This study also confirms a previous study by Ciarka *et al.* (2009), who reported that sunflower extracts are promoters at lower concentrations. Sharma and Satsangi (2013) found that higher concentrations (50-100%) of sunflower shoot aqueous extracts have significantly greater allelopathic potential than low concentrations of extracts.

It is concluded that results of the pot experiment on seed germination and early growth show that two months sunflower ground shoot has a greater effect on most parameters measured than aqueous sunflower shoot extract.

4.3.2 Effects of sunflower ground shoot and aqueous extract on soil properties

Soil is the environment where allelopathic activities happen. The effects of two month sunflower ground shoot and aqueous extract on soil properties were investigated. The present findings showed that two month sunflower ground shoot has more allelopathic influence on soil properties than two month sunflower aqueous shoot extracts.

Two month sunflower ground shoot at 9 g/pot significantly reduced calcium availability of soil in which *A. fatua* and *P. minor* were grown. Nevertheless, two month sunflower shoot aqueous extract did not significantly affect calcium availability. The present study also showed the effect of sunflower ground shoot and aqueous extracts on magnesium availability in soil. Two month sunflower shoot aqueous extract applied to soil at a concentration of 9% significantly decreased the availability of magnesium in *A. fatua* and *C. syriaca* soil. In contrast, no significant difference was recorded with the application of ground shoot.

Application of sunflower ground shoot to pots in which *A. fatua* and *P. minor* were grown significantly increased potassium availability in soil at all concentrations (3, 6, 9 g/pot), whereas two month sunflower shoot aqueous extract only increased potassium in soil in which *P. minor* was grown at the highest concentration (9%). In contrast, Chen *et al.* (2001) reported that incorporation of vanillin and p-hydroxybenzoic acid into woodland soil decreased the availability of potassium.

Soil pH was decreased by the application of two month sunflower ground shoot at the highest concentration (9 g/pot), whereas two month sunflower shoot aqueous extract did not significantly reduce soil pH. These results are in agreement with Souto *et al.* (2001), who indicated that allelochemicals may affect soil pH and therefore affect plant growth. Furthermore, allelochemicals are complex and susceptible to the effects of soil conditions (Kobayashi, 2004). It has been recognized that phenolic compounds may reduce soil pH (Sasikumar *et al.*, 2002; Zhang and Fu, 2009). A study by Inderjit and Dakshini (1994) showed that ground shoot of *Pluchea lanceolata* incorporated with soil (sandy-loam) significantly reduced pH. Eventually, the shoot straw of *P. lanceolata* significantly affected seed germination and seedling growth of rapeseed (*B. napus*).

Soil electrical conductivity was significantly reduced by applications of both two month sunflower ground shoot and aqueous extract at higher concentrations. In contrast, Inderjit and Dakshini (1994) reported that application of the leachate of *Pluchea lanceolata* in tomato soil increased electrical conductivity.

4.3.3 Effect of sunflower shoot residue and aqueous extract on chlorophyll a and chlorophyll b content

Khaliq *et al.* (2013) found that chlorophyll content was significantly reduced by the application of sunflower aqueous extract. On the other hand, Kamal and Bano (2009), investigated the effects of sunflower leaf, stem and root extracts on chlorophyll accumulation in two varieties of wheat seedlings (Margalla 99 and Chakwall 97) in a petri dish experiment and found that sunflower leaf and root aqueous extracts at a concentration of 1 g /9 ml distilled water significantly increased chlorophyll content in both varieties. Farhoudi *et al.* (2015) reported that sunflower extracts at higher concentrations reduced chlorophyll a and chlorophyll b content in leaves of Johnson grass. Our findings are also in agreement with results reported by Farhoudi and Lee (2012), who indicated that chlorophyll b content of wild mustard was significantly reduced by the effect of safflower extracts. It has been reported that phenolic compounds have allelopathic inhibitory effects on plant growth via influencing photosynthesis and chlorophyll content (Einhellig, 1995).

It is concluded that incorporation of sunflower two month ground shoot into soil and the application of sunflower two months shoot aqueous extract have similar inhibitory effect on chlorophyll content of crop and weed species.

Experiment 4

4.4.1 Identification and quantification of phenolic compounds present in sunflower shoot and root extracts from plants at different growth stages (one week, one month, two month and mature stage)

This study of identification and determination of phenolic compounds shows that in one month, two month and mature shoot extracts chlorogenic acid was the main phenolic acid with the highest concentration. The finding supports a previous study that reported that the concentration of chlorogenic acid is always higher than those of other phenolics (Wilson and Rice, 1968). These results are in agreement with Alsaadawi *et al.* (2012), who isolated thirteen allelochemical compounds, most of which were phenolic compounds, from sunflower extracts by using HPLC.

The present study supports the findings in terms of identification of phenolic compounds present in sunflower shoot and root extracts which were reported by Ghafar *et al.* (2001), who identified five phenolic compounds (chlorogenic, caffeic, syringic, vanillic and ferulic acids) in sunflower aqueous leaf extract, three (chlorogenic, ferulic and vanillic acids) from sunflower aqueous stem extract and one from sunflower aqueous root extract (ferulic acid). The study also indicated that sunflower aqueous extracts from leaves contained more total phenolic compounds than stems and roots.

It is concluded that, in the present study, sunflower shoot extracts contain more phenolic compounds than sunflower root extracts. Furthermore, it was observed that sunflower shoot and root at one week contain less phenolic compounds than one month, two month and mature stages. It can be seen that the highest concentration of total phenolic compounds was obtained from two month sunflower shoot extract while the lowest concentration was observed from one week root extract.

4.4.2 Effect of phenolic compounds on seed germination and early growth of *Brassica napus, Cephalaria syriaca, Triticum aestivum* and *Secale cereale*

According to the concentrations of phenolic compounds which had already been identified and quantified in two month sunflower shoot extract, the effects of phenolic compounds on seed germination and seedling growth of *B. napus*, *C. syriaca*, *T. aestivum* and *S. cereale* were investigated.

Our investigation of the effects of phenolic compounds (gallic acid, syringic acid, vanillic acid, protocatechuic acid, catechol, 4-hydroxybenzoic acid, p-coumaric acid, sinapic acid, ferulic acid, caffeic acid, chlorogenic acid, trans-cinnamic acid and total phenols) on seed germination and seedling growth of *B. napus, C. syriaca, T. aestivum* and *S. cereale* showed that seed germination and seedling growth were most sensitive to total phenolic compounds, followed by ferulic acid and chlorogenic acid.

The responses of seed germination and growth to phenolic compounds have been reported in previous studies which indicated that phenolic compounds contribute to the plant growth system and could affect seedling growth of plants through affecting plant hormones (Callaway and Aschehoug, 2000). Muscolo *et al.* (2001) investigated the effect of phenolic compounds on respiratory enzymes in seed germination of *Pinus laricio.* Their findings showed that the inhibition of seed germination was strongly correlated with inhibition of glycolytic enzyme activities.

Chlorogenic acid and ferulic acid had higher concentrations than other phenolic compounds which were quantified in the present study. Because phenolic compounds are found at different concentrations in sunflower extracts they are likely to have different effects on seed germination and growth. The findings indicated that the root parts of the studied plants are more sensitive to effects of phenolic compounds than the shoot parts in terms of dry weight and length. These findings are in accordance with reports that ferulic acid causes stress in plant roots and influences several physiological and biochemical processes, i.e. utilization of water, foliar expansion, root enlargement, photosynthesis, ion uptake, and respiration (dos Santos *et al*., 2008).

Experiment 5

4.5.1 Effect of the herbicide trifluralin at different concentrations on seed germination and seedling growth of *Brassica napus* and *Cephalaria syriaca*

The aim of this experiment was to find out the appropriate concentration of trifluralin in order to use it as a positive control with total phenolic compounds and sunflower extracts for further investigations. The effects of different concentrations of trifluralin on seed germination and early growth of *B. napus* and *C. syriaca* were tested in a petri dish experiment. The results indicated that seed germination percentage was sensitive to almost all concentrations but it was affected to the greatest extent by the highest concentration and least by the lowest concentration. Shoot length was also significantly reduced by all concentrations. Moreover, the greatest effect was with the highest concentration (900 ppm) and least with the lowest concentration (12.5 ppm). Although the greatest effect of trifluralin was recorded at 900 ppm, 100 ppm of trifluralin might be effective on seed germination and seedling growth of some weed species.

4.5.2 Investigations on the mechanism of herbicidal properties of aqueous extracts of sunflower shoots, their total phenolic compounds and the herbicide trifluralin on seed germination and early growth of some weed and crop species

The starting point of this study was based on the mode of action of aqueous extracts of sunflower shoots, total phenolic compounds and trifluralin on sugar content, protein content, proline content, DNA content, gibberellic acid, indole acetic acid and abscisic acid in *B. napus, C. syriaca, T. aestivum* and *S. cereale.*

4.5.2.1 Effect on sugar content

There is evidence that allelochemicals may exert an effect by influencing sugar metabolism in susceptible plants. Singh and Sunaina (2014) found that application of ferulic acid significantly reduced sugar content of tomato. Mohamadi and Rajaie (2009) found that aqueous eucalyptus (*Eucalyptus camadulensis*) extracts significantly reduced soluble sugar content in *Phaseolus vulgaris* and *Sorghum bicolor.* These findings are in agreement with Al-Taisan (2014), who investigated the allelopathic effect of leaf and root aqueous extracts of *Heliotropium bacciferum* at different concentrations (0, 10, 25, 50 and 75%) on soluble sugar content of *Oryza sativa* and *Teucrium polium* and found that soluble sugar content in shoot and root was

significantly affected by the increase in concentration of *H. bacciferum* extracts. It has been observed that the decrease in chlorophyll content leads to decreased photosynthesis and eventually decreased content of sugars, proteins, and amino acids (Singh and Rao, 2003). In complete contrast, however, Ahmad and Bano (2013) found that soluble sugar contents were significantly increased by the application of maize extracts on soybean.

4.5.2.2 Effect on protein content

Sunflower aqueous extract caused the highest increase in protein while total phenolic compounds caused the least increase in protein. Trifluralin significantly increased protein content in *S. cereale* and *T. aestivum.* These findings are in accordance with the findings by Verma and Rao (2006) that protein content of different varieties of *Glycine max* was increased by weed extracts from *Ageratum conyzoides* and *Solanum nigrum*. Further to the previous studies on protein content, Mali and Kanade (2014) indicated that aqueous leaf extracts of *Alternanthera sessilis* and *Cynodon dactylon* increased protein content to two or three times more than the control treatment in sorghum. Preston (2002) mentioned that synthetic herbicides interfere with enzymes or proteins that eventually influence the growth and metabolism of plant systems and allelochemicals may have similar mechanisms.

In contrast to the findings of this study, Kaur and Sharma (2015) reported that application of aqueous extracts of *Ageratum conyzoides* significantly decreased protein content of *Vigna radiata*. Also, Hussain *et al.* (2010) found that the herbicide pendimethalin and ferulic acid significantly reduced protein content in lettuce.

4.5.2.3 Effect on proline content

Proline is one of the osmoprotective molecules which protect organisms from stress and it is capable of accumulating in various organisms, such as invertebrates, bacteria, fungi and plants, through water stress and salinity (Abraham, 2004). Proline has also been recognised as a general stress indicator. Therefore, the content of this amino acid in plants may be indicative of allelopathic effects.

Phenolic compounds significantly reduced proline content in *S. cereale* and *C. syriaca,* while trifluralin only caused significant reduction in proline in *S. cereale*. No significant difference was observed between sunflower aqueous shoot extract and control treatments. It is notable that proline content was more sensitive to total phenolic compounds than to the application of trifluralin.

On the other hand, the results obtained are not in agreement with the results described by Kamal (2010), who found that sunflower allelochemicals significantly increased accumulation of proline in wheat (*Triticum aestivum*). Further to the previous information Kamal (2010) also found that aqueous extract from sunflower leaves had a greater effect and increased free proline more than stem and root aqueous extracts in two varieties of wheat (Margalla 99 and Chakwall 97). Moreover, Das *et al.* (2012) found that aqueous leaf leachates of seven tree species (*Acacia auriculiformis*, *Anacardium occidentale*, *Albizia lebbeck*, *Eucalyptus citriodora*, *Emblica officinalis*, *Shorea robusta* and *Tectona grandis*) significantly increased proline content in *Cicer arietinum*. Durán-Serantes *et al.* (2002) also reported that allelochemicals (2-benzoxazolinone, p-hydroxybenzoic acid, and ferulic acid) and herbicides (linuron and fluometuron) affected free proline accumulation in *Dactylis glomerata*. Furthermore, the study also mentioned that the two herbicides almost doubled free proline.

4.5.2.4 Effect on DNA content

All applications reduced DNA content in the monocotyledons *S. cereale* and *T. aestivum*, whereas no significant difference was recorded between the treatments and control in the dicotyledons *B. napus* and *C. syriaca.* These results are similar to the results obtained by Mohamed and El-Ashry (2012), who observed that aqueous extract of rapeseed (*Brassica nigra*) at 0.25, 0.50 and 1% concentrations significantly inhibited cell division and increased the percentage of chromosomal aberrations in mitotic and meiotic cell divisions of pea (*Pisum sativum*).

Further to the previous findings, Padhy *et al.* (2000) studied the effects of different concentrations (5, 10, l5 and 20%) of aqueous leachates of *Eucalyptus globulus* on physiological and biochemical processes of finger millet (*Eleusine corocana*). The study showed that all concentrations significantly decreased total DNA and RNA contents of shoots and roots.

Allelochemicals are associated with the inhibition of cell division through effects on mitosis, chromatin organization, and DNA physical and chemical properties (Zhang *et al.*, 2010; Teerarak *et al.*, 2012). Seigler (1996) reported that allelochemicals influence nucleic acids and consequently affect DNA modification.

4.5.2.5 Effect on plant hormones (GA, IAA, and ABA)

The results from testing the effects of sunflower shoot aqueous extracts, their total phenolic compounds and trifluralin on plant hormones show that GA was affected by application of all treatments only in *B. napus*. Sunflower shoot aqueous extract and total phenolic compounds caused greater reduction of ABA and IAA than application of trifluralin. Similar results were obtained by Kamal (2010), who studied the effects of sunflower extracts on plant hormone contents (IAA, GA and ABA) of two wheat varieties (Margalla 99 and Chakwall 97) in a petri dish experiment. The findings showed that sunflower aqueous extract (leaves, stems, and roots) significantly decreased IAA and GA. However, ABA content in wheat seedlings was significantly increased.

Allelochemical influences on plant growth are implicated in control of plant hormone levels. This involvement could represent an essential factor affecting regulation of numerous metabolic processes which control plant growth (Olofsdotter, 1998). Previous studies revealed that plant hormones can be regulated by allelochemicals. Moreover, secondary metabolites influence phytohormones (gibberellins and auxins) and significantly affect cell elongation in plants (Sharp *et al.*, 2000; Figueiredo *et al.*, 2011; Gamalero and Glick, 2011). Another study regarding the effect of allelochemicals on plant hormones which supports our findings was by Kefeli and Turetskaya (1968), who showed the effect of allelochemicals on plant hormone activity. The study indicated that some phenolic compounds extracted from aqueous extracts of some weed plants have inhibitory effects on the activity of IAA and gibberellin (GA).

Further to the previous findings, Balah and Latif (2013) studied the bioherbicidal effects of aqueous extracts of medicinal plants, *Thymus vulgaris, Salvia officinalis* and *Calendula officinalis,* on plant hormonal content of wheat (*Triticum aestivum*) and its associated weeds *Lolium multiflorum* and *Phalaris paradoxa* under laboratory conditions. The results showed that aqueous extracts of *Thymus vulgaris* reduced IAA content of wheat seedlings but GA and ABA content were reduced in *Calendula officinalis.*

4.5.3 Effect of sunflower ground shoot, its total phenolic compounds and trifluralin on seed germination and growth of *Secale cereale*, *Triticum aestivum*, *Brassica napus*, and *Cephalaria syriaca* in pot experiment

In this experiment, the effects of sunflower ground shoot (9 g/pot), its total phenolic compounds (15 ml/pot of 26.5 mg/100 ml distilled water), and trifluralin (13 ml/pot based on 2.5 L of 480 g/L active ingredient per hectare) on seed germination and seedling growth of *S. cereale*, *T. aestivum*, *B. napus* and *C. syriaca* were investigated in a pot experiment.

The findings show that trifluralin had the greatest effect on seed germination, shoot and root length, and shoot and root dry weight while total phenolic compounds had the least effect. Total phenolic compounds and sunflower ground shoot had no significant effect on root dry weight of any plant species. Reigosa *et al.* (1999) reported that physiological and ecological effects of using herbicides are stronger than using allelochemicals. However, opposite studies was observed by Leather (1987), who showed that the interference of sunflower plants with weed seed germination had the same effect as using commercial herbicides.

It is concluded that incorporation of sunflower ground shoot has a greater inhibitory effect than total phenolic compounds equivalent to those in two months shoot extract. This could be because these total phenolic compounds do not represent all phenolic compounds in sunflower ground shoot: there might still be some more phenolic compounds or other allelochemicals that have not been identified and quantified yet. Indeed, Macías *et al.* (2002b) isolated about 125 natural allelochemical compounds from different sunflower extracts, including phenolics, triterpenes, steroids, flavonoids, heliespirones, heliannuoles, sesquiterpenes and helikauranoside A (Macías *et al.*, 2002a).

CHAPTER 5

CONCLUSIONS AND FURTHER WORK RECOMMENDATION

5.1 Conclusion

Our investigations of the effects of sunflower shoot and root aqueous extracts at different growth stages on seed germination and growth of some crop and weed species indicated that sunflower shoot extract from two month old plants has a greater influence on seed germination and seedling growth than other extracts. However, one month root extract turned out to have the most negative impact on seed germination and seedling growth in comparison with root extracts from other growth stages of sunflower. The most effective sunflower aqueous shoot extract had a greater influence than the most effective sunflower aqueous root extract.

Laboratory experiments were carried out in order to find out at what concentration sunflower shoot aqueous extract causes the highest negative impact on germination and seedling growth of the studied plants. Both seed germination and seedling growth were completely inhibited at the highest concentrations (5% and 10%) of aqueous shoot extract (mature stage) tested. It is concluded that low concentrations may not significantly inhibit germination and early growth of plants. Testing the effect of sunflower shoot extract on mitotic index gave no significant difference between control and test treatments. However, sunflower shoot extract significantly reduced cell elongation.

As it had the highest allelopathic potential, two month sunflower shoot extract was chosen for further study. Pot experiments were carried out to investigate the effects of two month sunflower shoot aqueous extract and ground shoot on seed germination and seedling growth, soil properties and chlorophyll content. It has been concluded that two month sunflower ground shoot has a greater effect on most parameters measured than aqueous sunflower shoot extract. However, two month ground shoot and two month sunflower shoot extract had similar effects on chlorophyll content and electrical conductivity of soil in which test species were grown.

Using HPLC for identification and determination of phenolic compounds present in sunflower shoot and root samples, twelve phenolic compounds were identified from sunflower shoot extracts while four phenolic compounds were identified from sunflower root extracts. The

highest concentration of total phenolic compounds was found in two month sunflower shoot extract and the lowest concentration was observed in sunflower one week root extract.

Plant age can influence amounts of allelopathic compounds produced. From the experiment regarding identification and determination of phenolic compounds, it is evident that two month aqueous sunflower shoot extract contains higher amounts of phenolic compounds than extracts from other growth stages. It was likely that the differences in amounts of phenolic compounds would at least partly explain why aqueous shoot and root extracts from sunflower plants of different ages had different effects on germination and growth of some crop and weed species. Therefore, according to the concentrations of phenolic compounds which had already been identified and quantified in two month sunflower shoot extract, the effects of phenolic compounds on germination and seedling growth were investigated, both individually and in combination. It has been concluded that seed germination and seedling growth were most sensitive to total phenolic compounds, while ferulic acid and chlorogenic acid were the most effective individual compounds. It is also worth mentioning that the root part of the studied plants is more sensitive to effects of phenolic compounds than the shoot part in terms of dry weight and length.

The highest effect of the herbicide trifluralin was at a concentration of 900 ppm but 100 ppm of trifluralin might affect seed germination and seedling growth of some weed species. Results from the evaluation of the effects of sunflower two month ground shoot, total phenolic compounds and trifluralin in the pot experiment indicated that trifluralin has the highest effect on seed germination and seedling growth while total phenolic compounds have least effect. However, the applied concentration of trifluralin was higher than the concentration of total phenolic compounds. Since incorporating sunflower ground shoot into soil gives better allelopathic potential than the application of total phenolic compounds, the total phenolic compounds that were extracted from shoots and identified do not completely explain the allelopathic potential of shoots.

Several physiological parameters were studied in seedlings in order to give insight into how aqueous extracts of sunflower shoots and their total phenolic compounds affect physiological functions of plants, in comparison to trifluralin. The results depended on the physiological parameter. Trifluralin had the greatest effect on sugar content, whereas total phenolic compounds lowered proline content more than trifluralin and sunflower aqueous extract. Sunflower aqueous extract caused the highest increase of protein while total phenolic compounds caused the least increase of protein. Sunflower shoot aqueous extract and total

phenolic compounds caused greater reduction of ABA and IAA than application of trifluralin. Overall, the physiological parameter that most closely corresponds to effects on plant growth seems to be sugar content.

In summary, the major key findings are:

1. Two month sunflower shoot aqueous extract has a greater influence on seed germination and seedling growth than other extracts (one week, one month and mature stage).
2. Two month sunflower shoot aqueous extract contains more phenolic compounds than extracts from other growth stages (one week, one month and mature stage).
3. Incorporating sunflower ground shoot into soil gives better allelopathic potential than the application of sunflower shoot aqueous extract.
4. Incorporating sunflower ground shoot into soil also has more allelopathic influence than total phenolic compounds.
5. At the concentrations tested, trifluralin gives a more negative influence on growth and physiological activities than sunflower shoot aqueous extract and total phenolic compounds.
6. The results give high encouragement to transferring the technique from pots to the field.

Therefore, our investigations regarding sunflower shoot and root aqueous extracts at different growth stages on seed germination and seedling growth indicated that sunflower shoot extract at two months old has a greater negative impact than other extracts. Consistent with this finding, sunflower two month shoot extract contains higher amounts of phenolic compounds than extracts from other growth stages. To our knowledge, this is the first attempt to test the roles of phenolic compounds from sunflower extracts by quantifying them and studying the effects of the same concentrations that are present in the extracts.

From the extensive investigation of the allelopathic effect of two months sunflower ground shoot and sunflower aqueous shoot extract in greenhouse experiments, our findings provide evidence, for the first time, that incorporation of sunflower ground shoot with soil gives more allelopathic influence on germination and seedling growth than the application of sunflower aqueous shoot extract.

A conclusion can be drawn that comparisons of the mechanism of herbicidal action of aqueous extracts of sunflower shoots, total phenolic compounds and trifluralin indicate that trifluralin has a greater effect on physiological activities than other treatments. Furthermore, total phenolic compounds reduced proline content more than trifluralin and sunflower ground shoot. Furthermore, sunflower shoot aqueous extract increased protein amount more than other applications, whereas trifluralin reduced protein content. Thus, trifluralin and sunflower shoot extract have different effects on plant functions. Since sunflower shoot aqueous extract and total phenolic compounds reduce the amount of ABA and IAA, part of their allelopathic effect on plant growth may be through reducing concentrations of hormones that are required for growth and causing imbalances in amounts in plants. Thus, this involvement could represent an essential factor affecting regulation of numerous metabolic processes.

5.2 Further work and recommendations

From this study, some recommendations can be made.

The phenolic compounds that have been identified and tested so far do not completely explain the allelopathic potential of shoots. HPLC chromatograms show that there are still a number of unidentified phenolic compounds in extracts. Some of these unidentified compounds may have allelopathic effects. Therefore, more standards should be used for identification and determination of phenolic compounds in sunflower shoot and root extracts, in order to cover as many peaks as possible and to give a more complete picture of the phenolic compounds in the extracts.

Also further experiments are needed to investigate the effects of different concentrations of individual phenolic compounds found in sunflower extracts on germination and seedling growth. The concentrations that have been used for this study were based on what was identified and quantified from sunflower shoot and root extracts, in which some phenolic compounds were found in very low concentrations.

For weed control based on allelopathy to be effective, it must work in the field. The results of the present study provide a strong basis for field trials, but we cannot be certain whether extracted phenolic compounds, unfractionated aqueous extracts or ground shoots will be most effective in the field. Therefore, further experiments are also recommended to study the effects of total phenolics, sunflower aqueous extracts at different concentrations and sunflower ground shoots in the field.

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CHAPTER 7 APPENDIX

7.1 HPLC chromatogram for phenolic compounds

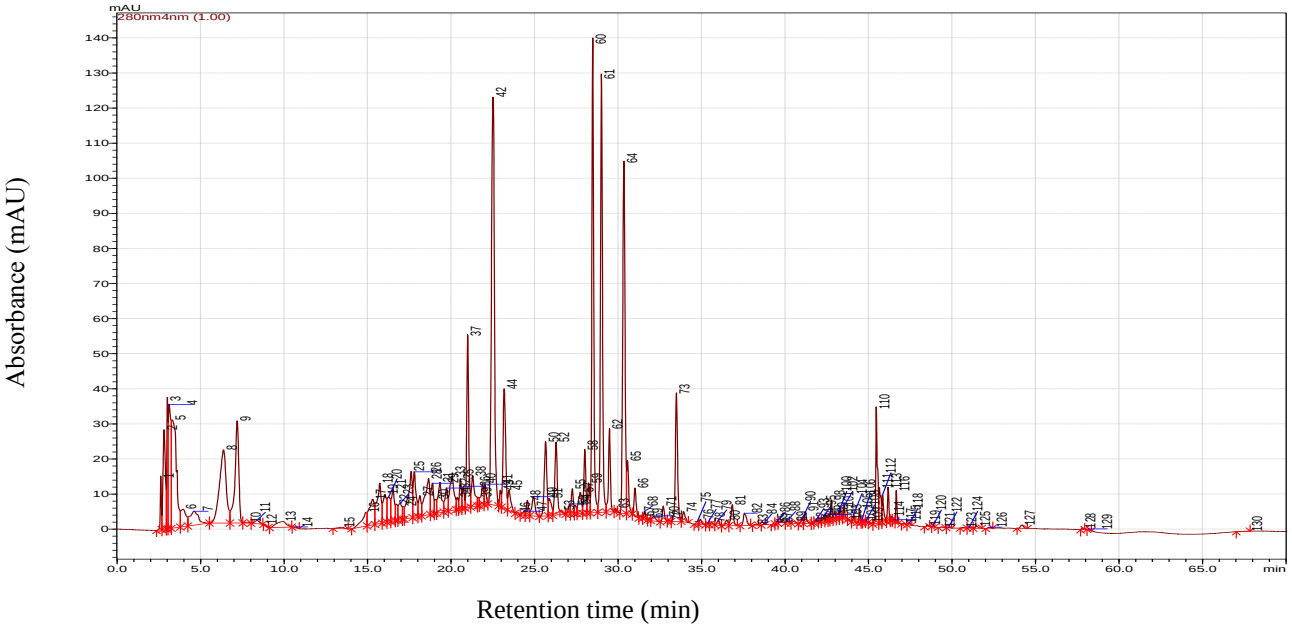

Figure 103 HPLC chromatogram recorded at 280 nm with diode array detector of phenolic compounds in sunflower root extract (one week).

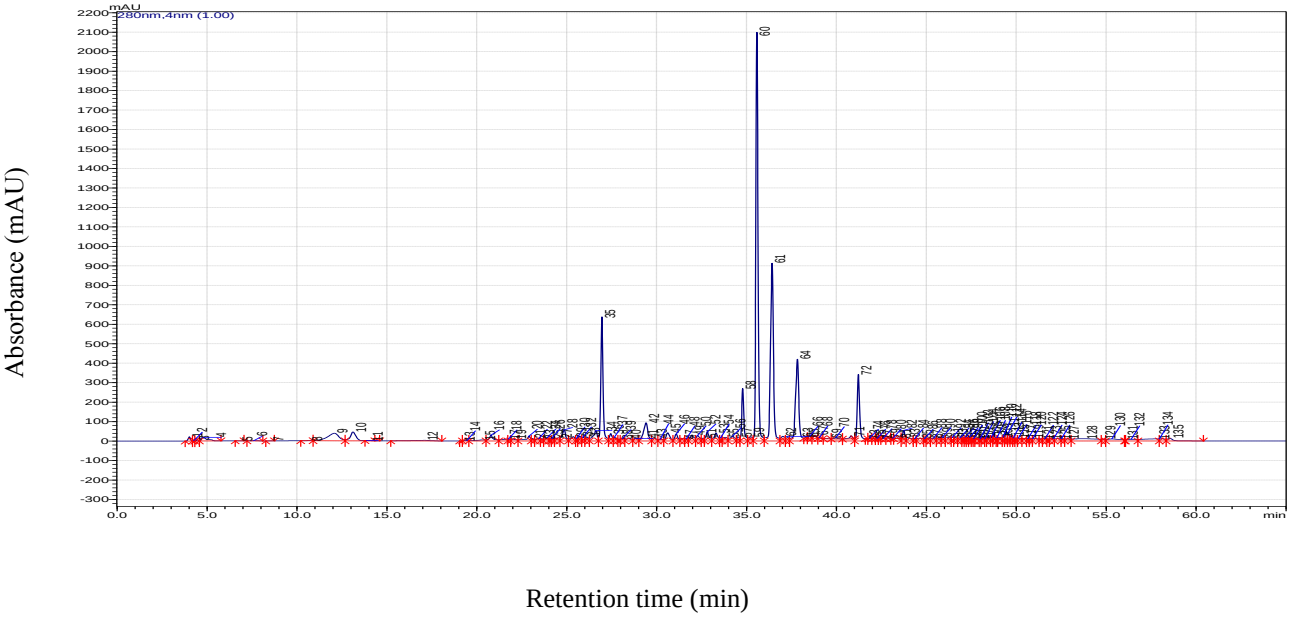

Figure 104 HPLC chromatogram recorded at 280 nm with diode array detector of phenolic compounds in sunflower root extract (one month).

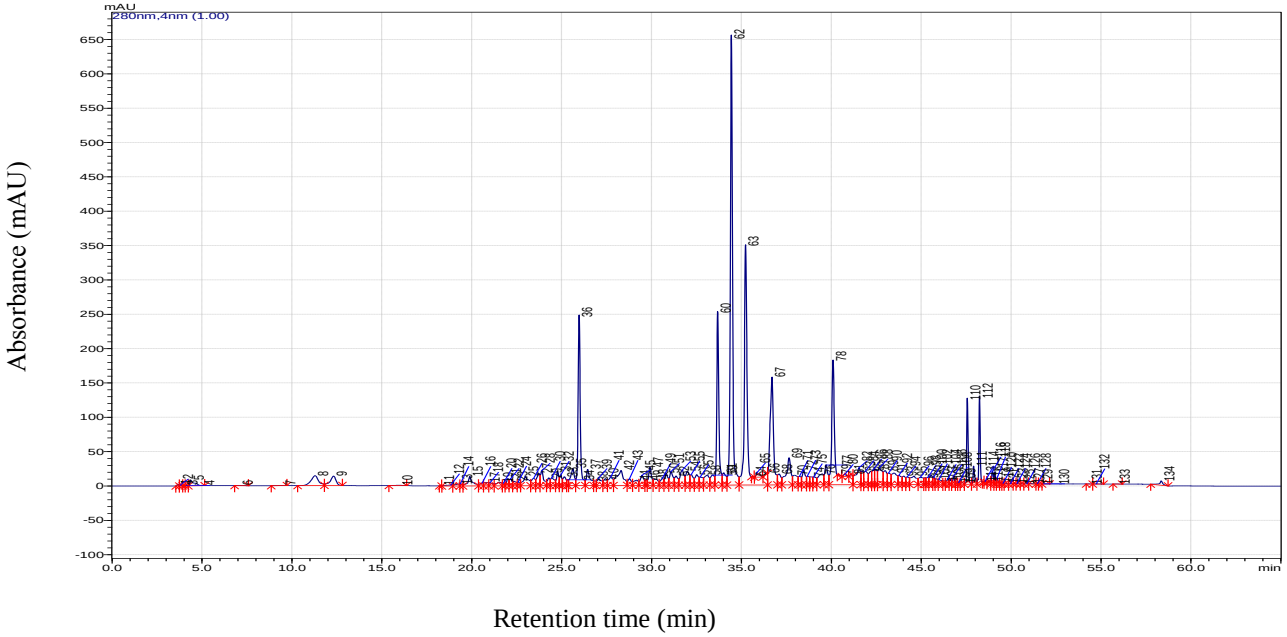

Figure 105 HPLC chromatogram recorded at 280 nm with diode array detector of phenolic compounds in sunflower root extract (two month).

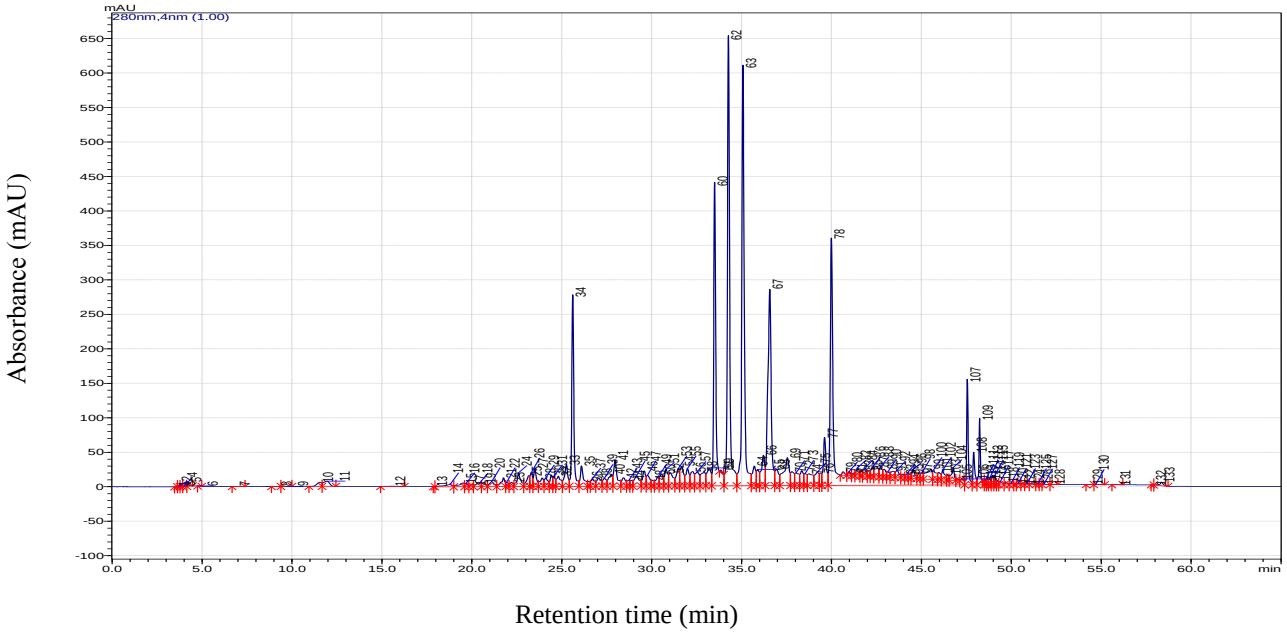

Figure 106 HPLC chromatogram recorded at 280 nm with diode array detector of phenolic compounds in sunflower root extract (mature stage).

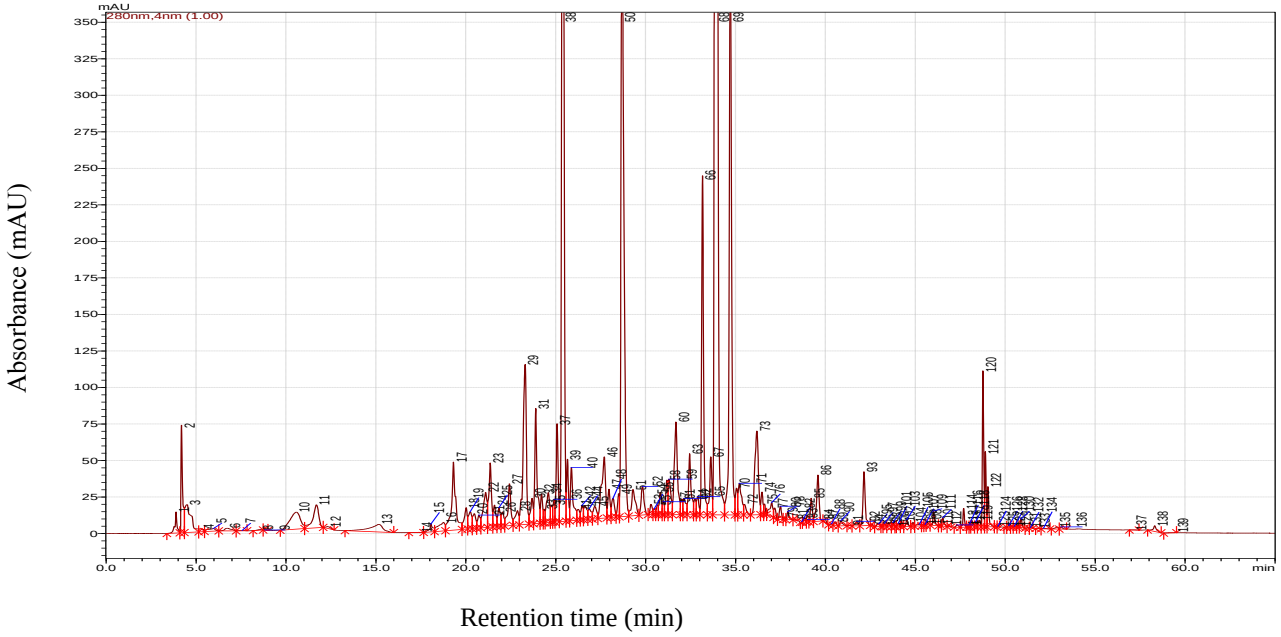

Figure 107 HPLC chromatogram recorded at 280 nm with diode array detector of phenolic compounds in sunflower shoot extract (one week).

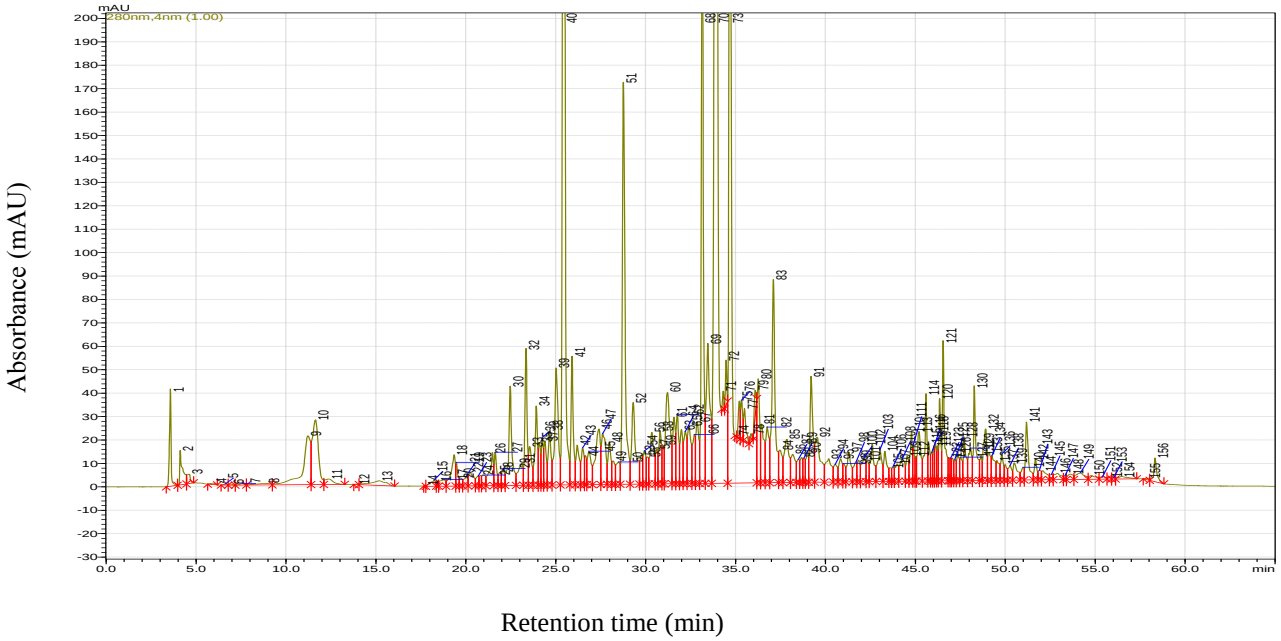

Figure 108 HPLC chromatogram recorded at 280 nm with diode array detector of phenolic compounds in sunflower shoot extract (one month).

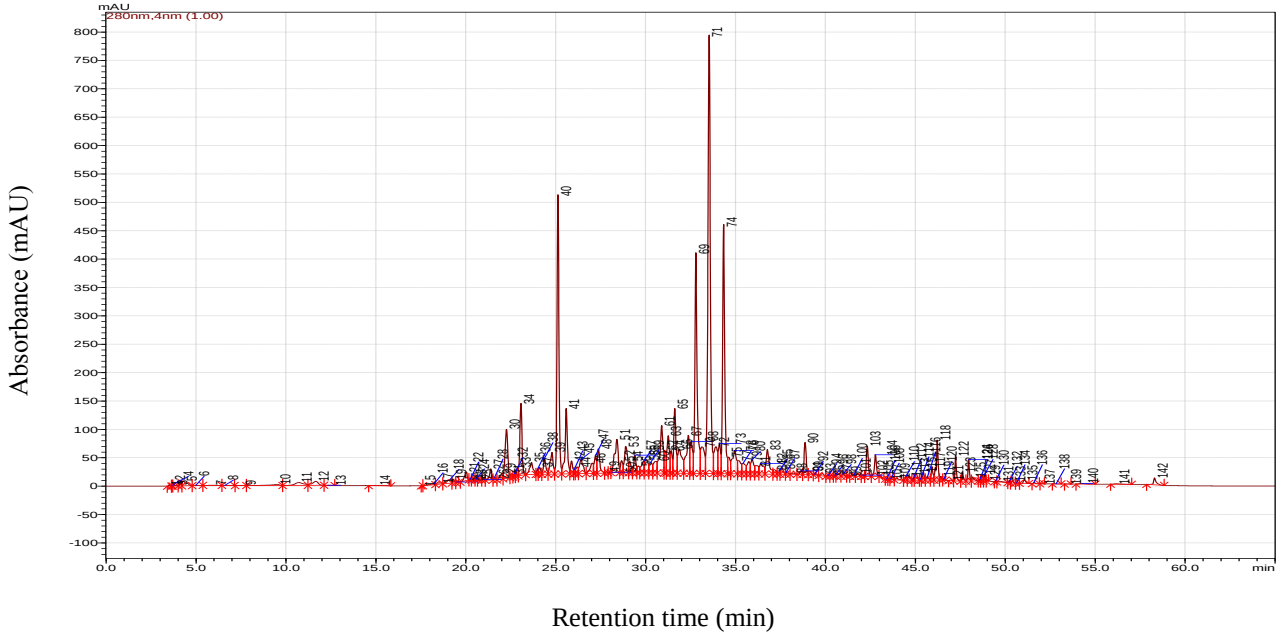

Figure 109 HPLC chromatogram recorded at 280 nm with diode array detector of phenolic compounds in sunflower shoot extract (two month).

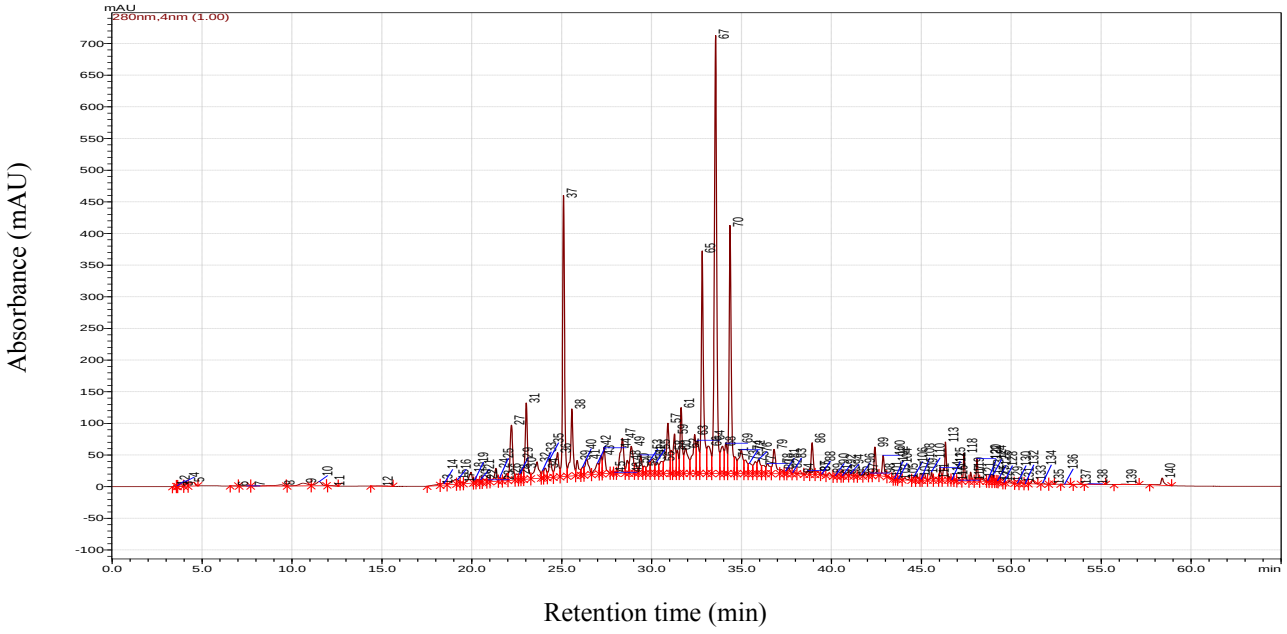

Figure 110 HPLC chromatogram recorded at 280 nm with diode array detector of phenolic compounds in sunflower shoot extract (mature stage).

7.2 HPLC chromatogram for chlorophyll analysis

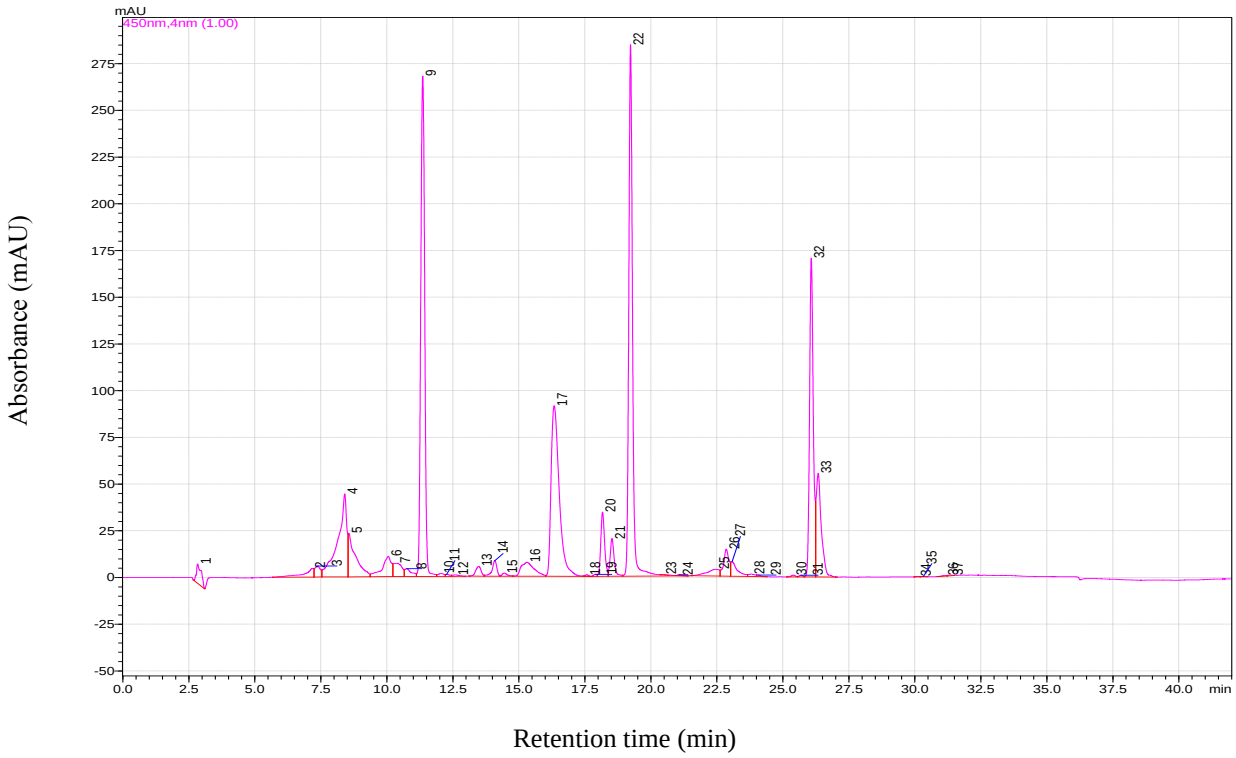

Figure 111 HPLC chromatogram recorded at 450 nm with diode array detector of chlorophyll in *Avena fatua* (control).

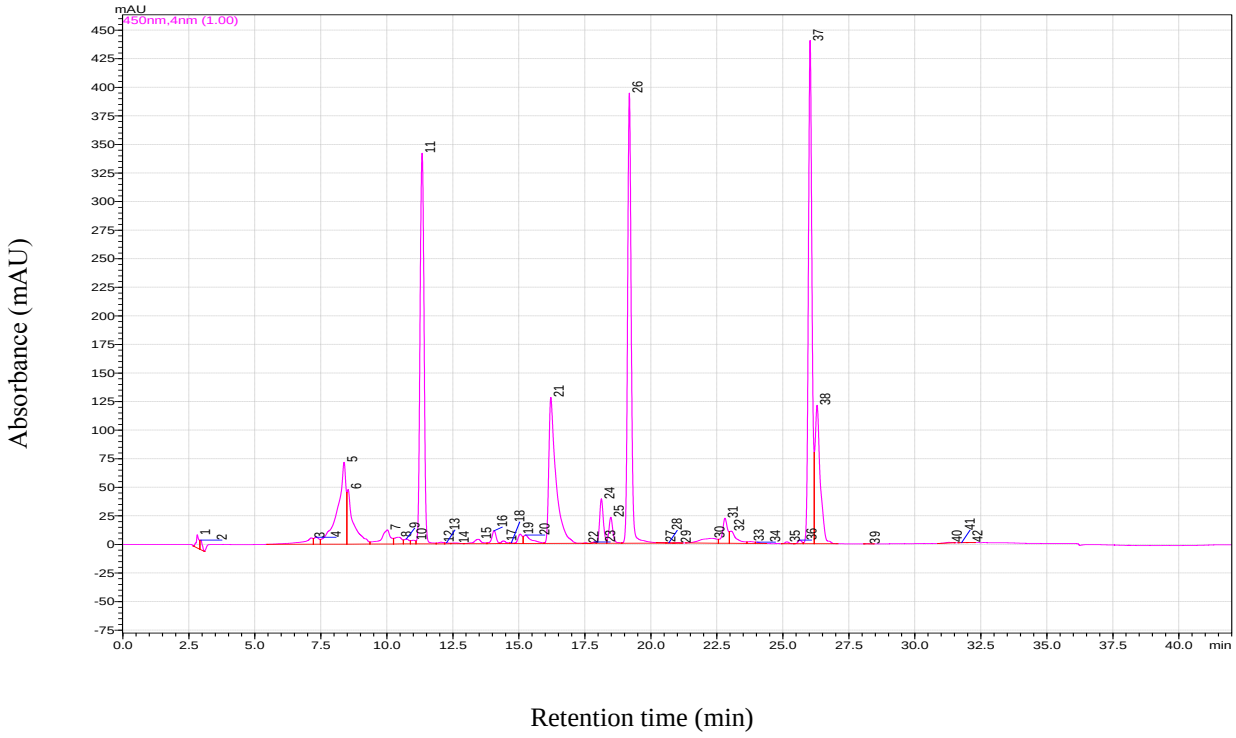

Figure 112 HPLC chromatogram recorded at 450 nm with diode array detector of chlorophyll in *Avena fatua* (3% aqueous extract).

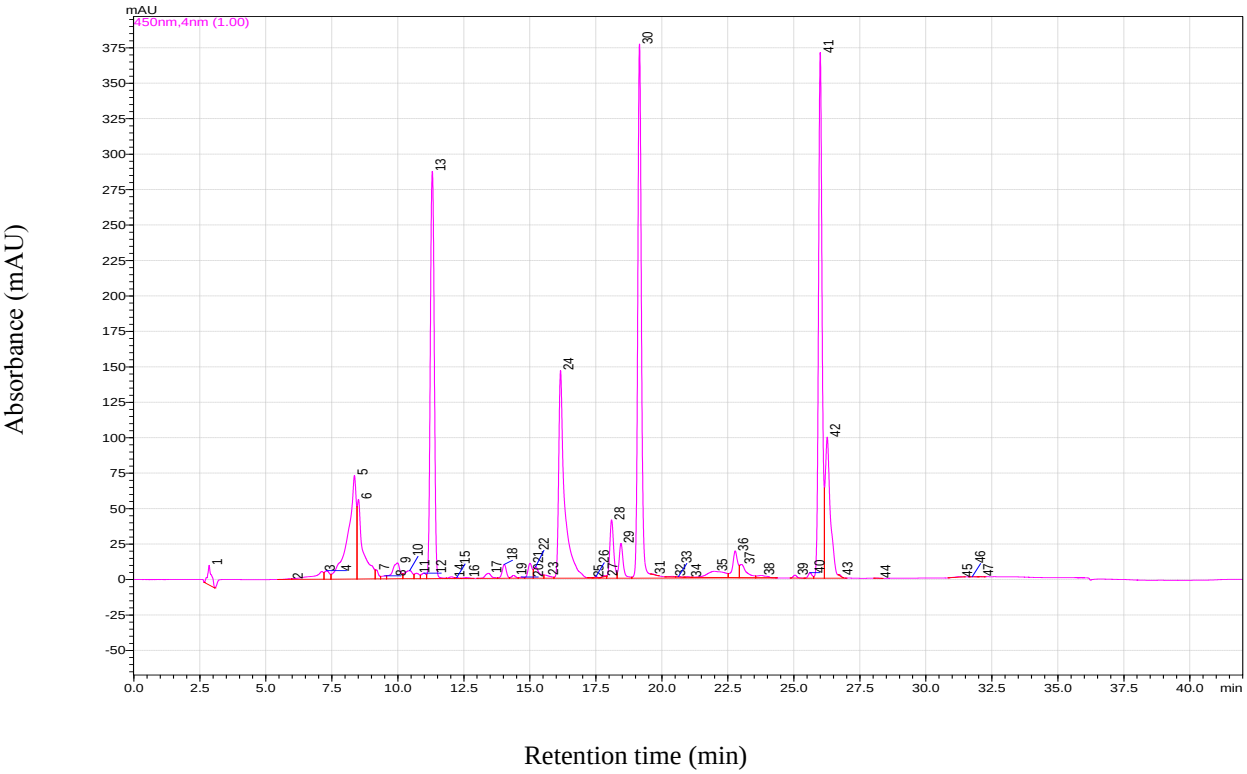

Figure 113 HPLC chromatogram recorded at 450 nm with diode array detector of chlorophyll in *Avena fatua* (6% aqueous extract).

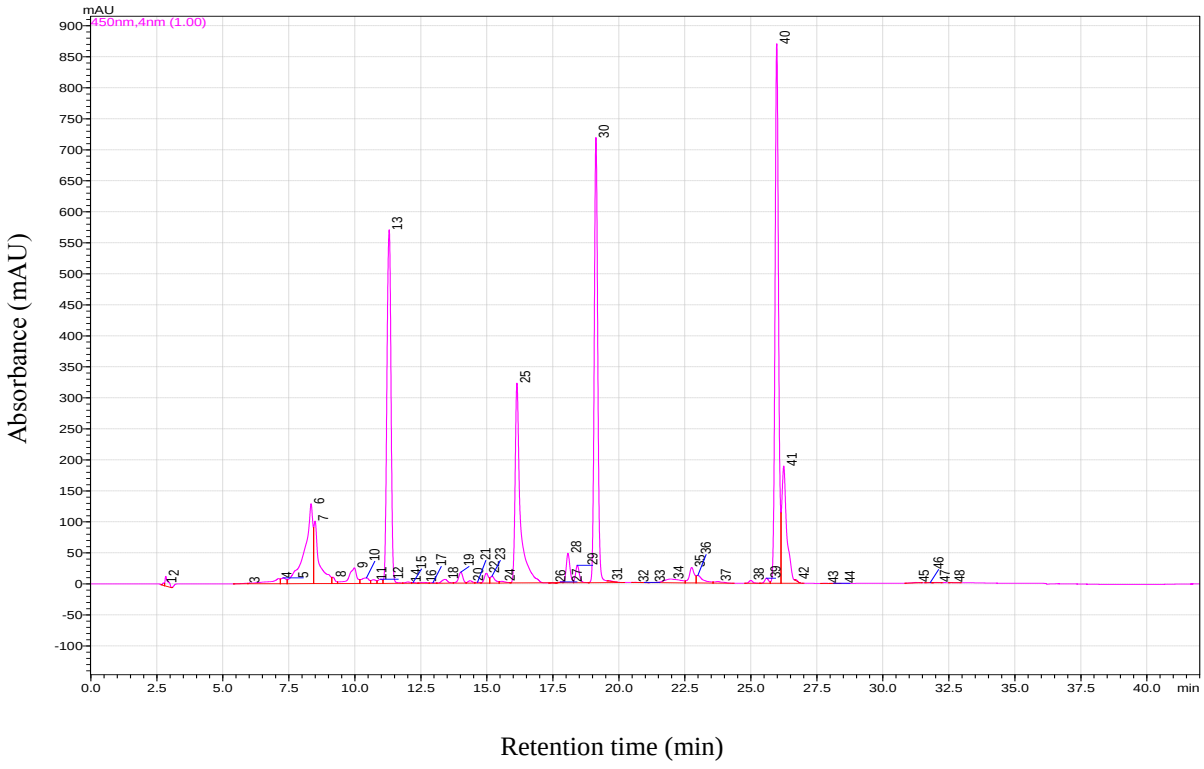

Figure 114 HPLC chromatogram recorded at 450 nm with diode array detector of chlorophyll in *Avena fatua* (9% aqueous extract).

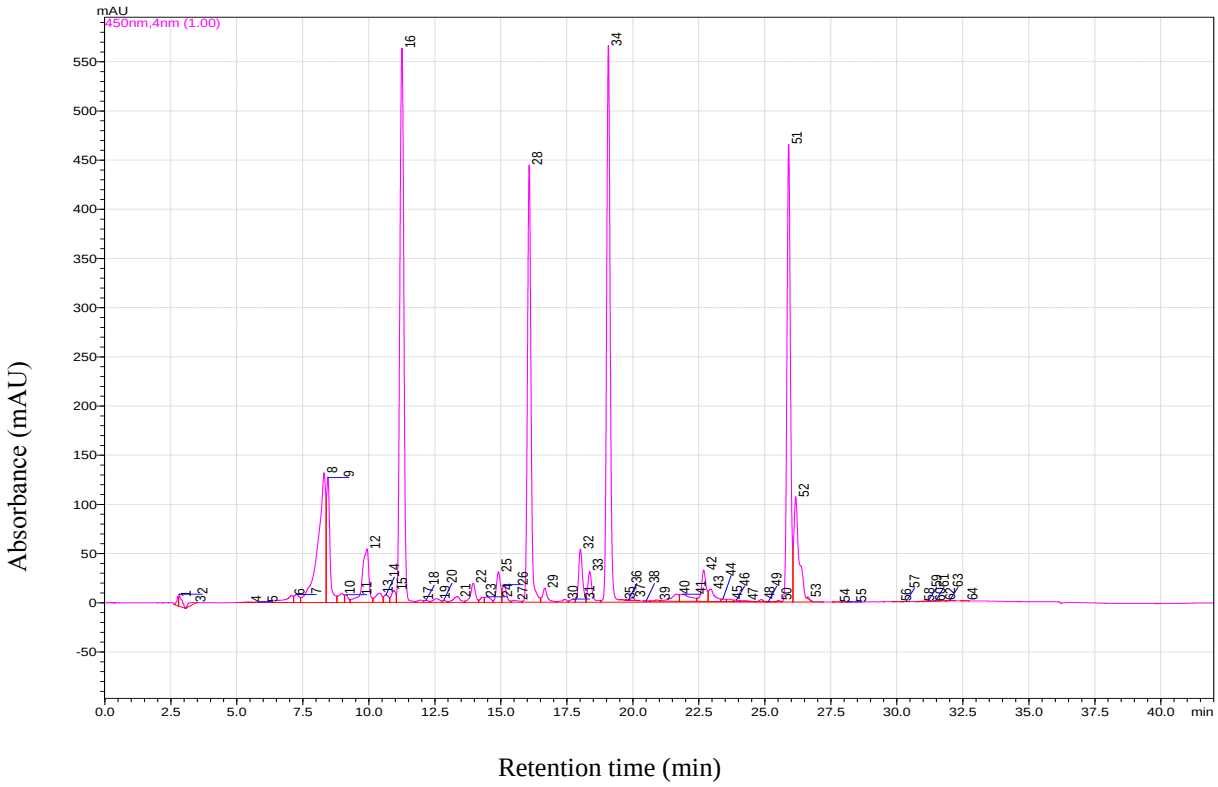

Figure 115 HPLC chromatogram recorded at 450 nm with diode array detector of chlorophyll in *Avena fatua* (3g/ pot ground shoot).

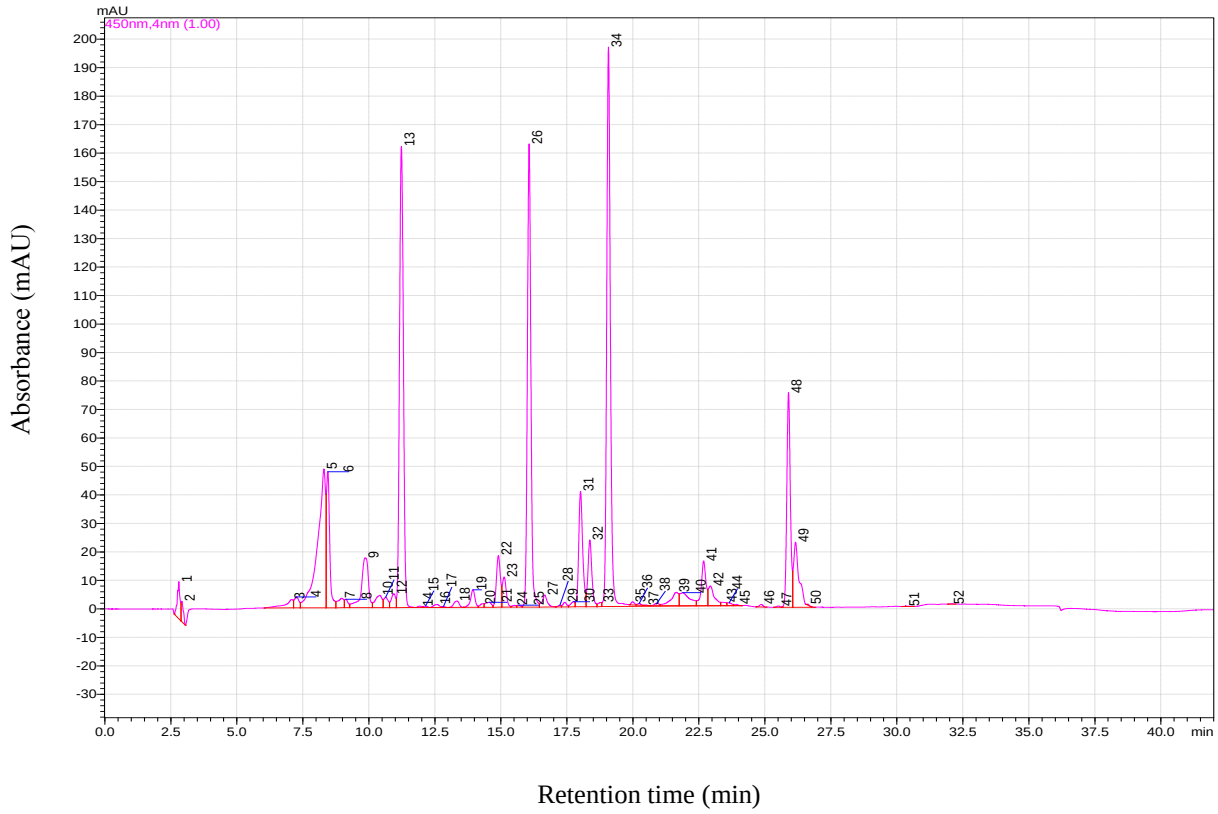

Figure 116 HPLC chromatogram recorded at 450 nm with diode array detector of chlorophyll in *Avena fatua* (6 g/pot ground shoot).

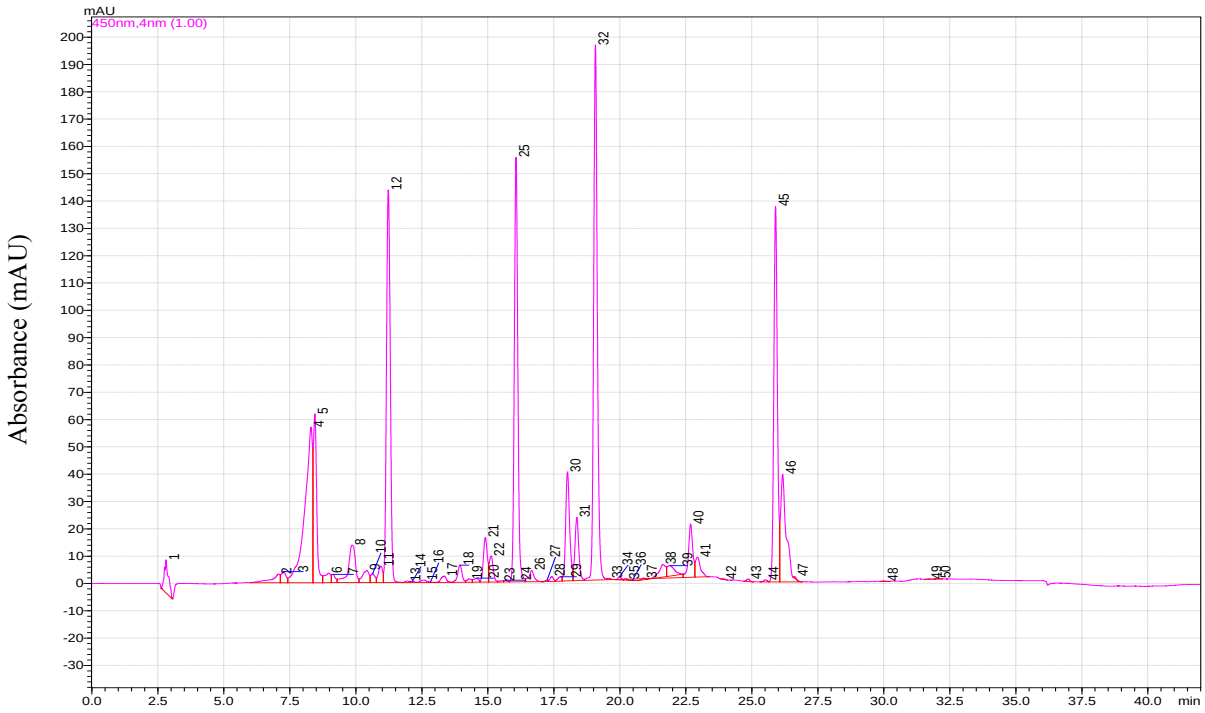

Figure 117 HPLC chromatogram recorded at 450 nm with diode array detector of chlorophyll in *Avena fatua* (9 g/pot ground shoot).

7.3 The chi-square statistic of mitotic phases (prophase, metaphase, anaphase, telophase)

Table 6 Chi square statistic of mitotic phases.

Results					
	Prophase	Metaphase	Anaphase	Telophase	*Row Totals*
Control	24 (27.36) [0.41]	19 (21.77) [0.35]	21 (19.54) [0.11]	27 (22.33) [0.98]	91
Test	25 (21.64) [0.52]	20 (17.23) [0.45]	14 (15.46) [0.14]	13 (17.67) [1.23]	72
Column Totals	49	39	35	40	163 (Grand Total)

The chi-square statistic is 4.1882. The P value is 0.241843. The result is *not* significant at $P < 0.05$.