ALLELOPATHIC EFFECT OF *CISSUS CACTIFORMIS* EXTRACT ON SEED GERMINATION AND SEEDLING GROWTH OF LETTUCE

BY

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DECLARATION

I, Makoena Flora Sebola, do hereby declare that this research mini-dissertation report submitted to the University of Limpopo, for the Master of Science degree in Agriculture (Plant Production) has not previously been submitted by me for a degree at this or any university; it is my work in design and in execution, and all material contained herein has been dully acknowledged.

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DEDICATION

I would like to dedicate this mini-dissertation to my parents (Shubert and Agnes Sebola), my sister and her son (Nare and Lethabo Sebola) and to my late grandmother Fridah Sebola.

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ABSTRACT

Following the withdrawal of synthetic pesticides from agro-chemical markets, several extracts from various plants have been tested. Despite this progress, some plant extracts with bio-pesticidal properties have also been documented for their phytotoxic properties. This study was conducted to investigate allelochemical content of *Cissus cactiformis* plant. Also, to determine its allelopathic effect based on the presence of the phytochemical components on seed germination and seedling growth of lettuce. Phytochemical analysis of *C. cactiformis* plant extract showed differences in the allelochemical components of different extracts tested. Saponins, terpenoids, flavonoids, tannins, alkaloids, coumarins, phytosterols were detected in plant materials extracted using acetone, methanol and ethanol. With these extracts the presence of phenolic compounds and cardiac glycoside were not detected. However, in distilled water extract terpenoids, flavonoids, tannins, alkaloids, coumarins and phenolic compounds were detected, with saponins and cardiac glycoside being absent. Lettuce seeds in germination bioassay were exposed to four treatments, *viz*. 0, 0.5, 1.0 and 1.25mg/mℓ of the different extract laid-out in a completely randomised design replicated four times. In all variables measured, treatments were significantly ($P \leq 0.01$) different for acetone, ethanol, distilled water and methanol extract of *C. cactiformis* plant. Mean germination percentage, hypocotyl and radicle length for control were highest when compared to other levels of each extract type. Similarly seedling growth bioassay comprised of six treatments, viz. 0, 0.5, 1.0, 1.25, 2.0 and 2.25 g extracts of *C. cactiformis* plant per pot, arranged in a randomised complete design, with six replicates in the greenhouse. All measured variables were not significant at $P \leq 0.05$ which showed no variation. The results revealed that irrespective of the solvents used, extract of *C. cactiformis* plant had allelopathic effects on the seed germination whereas no effect was observed on seedling growth of lettuce which makes *C. cactiformis* plant possible to be adapted as alternative post-emergent bio-nematicide without affecting the growth of other crops.

CHAPTER 1
GENERAL INTRODUCTION

1.1 Background

Since the introduction of allelo-chemistry as the alternative phytochemical-based strategy for nematode control (Chitwood, 2002), several researchers have tested this idea using various plants. Allelo-chemistry is the direct influence of chemicals released from one plant and affects the development and growth of another plant (Rice, 1989). Recently, Khosa (2013) evaluated extract of *Cissus cactiformis* plant (Figure 1.1) extract against *Meloidogyne incognita* and yielded promising results. However, when this material was applied at planting using (Mashela, 2002) recommended dosages resulted in total seed germination and seedling growth failure (unpublished data).

This General introduction focuses on the (1) background, which includes the description of the problem, its impact, possible causes of the problem and the proposed solution, (2) problem statement, (3) motivation of the study, (4) the objectives, (5) the hypotheses and (6) the format of the thesis.

Figure 1.1 Picture of *Cissus cactiformis* plant (Photo by Khosa, 2013.

1.1.1 Description of the research problem

Secondary metabolites from other plants, which conferred different pesticidal activities on these plants, also showed they interfered with crop plants (Duke *et al.,* 2000; Mafeo, 2012). Allelochemicals interfere with crop growth through lack of root hairs and swelling or necrosis of root tips and curling of the root axis (Ayeni *et al.,* 1997). Also, they increase the number of seminal roots, discolouration of plant leaves, reduce dry weight accumulation and lower reproductive capacity (Rice, 1989; Yu and Matsui, 1994). Apart from the scantily documented medicinal uses of the *C. cactiformis* plant, little is known about the allelopathic effect of this plant extract on germination and seedling growth of other crop plants. Also, other plants that conferred different pesticidal activities also possess allelopathic effects on crop plants (Khanh *et al*., 2005). It would be ideal to determine allelopathic activities of *C. catiformis* plant extract prior to its adaption as a pre-emergent bio-nematicide.

1.1.2 Impact of the research problem

Allelochemicals suppressing or eliminating competing plant species are interesting sources of natural pest, pathogen and weed protectants. Unlike synthetic chemicals, allelochemicals have proven nature specificity, biodegradability, low toxicity and pollution-free (Lee *et al.,* 1997; Anaya, 1999). Generally, the registering agricultural agencies require much less research data to register a phytochemical than to register conventional pesticides (Chitwood, 2002). Again, their registration costs are usually lower. There are also some limitations in using allelochemicals potentiality, which include their undesirable effects on non-targeted plant species and the existence of many abiotic and biotic soil factors that influence phytotoxic levels of allelochemicals (Huang *et al.,* 1999; Inderjit *et al.,* 1999). When released into the soil, the secondary plant compounds inhibit or stimulate germination, seedling growth and establishment of other crops (Abu-Romman *et al.,* 2010).

1.1.3 Possible cause of the research problem

Secondary compounds with allelochemical potential have great chemical diversity and are involved in many physiological, metabolic and ecological processes (Anaya, 1999). Allelochemicals with effect on other crop might be due to direct or indirect phytotoxicity through mediation of the soil environment (Suman *et al*., 2002). Allelopathic inhibition or stimulation process is complex and can involve the

interaction of different classes of chemicals and their movement in the soil, transportation, absorption and mode of action are not well understood (Rimando *et al.,* 2001).

1.1.4 Proposed solution

Plant extracts are important sources of new agricultural chemicals. Previous studies showed evidence that various plant species posses antifungal, nematicidal and antibacterial activities (Mashela, 2002; Maji *et al.,* 2005). Recently, *C. cactiformis* plant extract has been identified as being an effective bio-nematicide (Khosa, 2013). Thus, it is important to know if this plant would not posses negative allelopathic effects prior to its adaption as alternative bio-nematicides.

1.2 Problem statement

Following the withdrawal of synthetic pesticides due to their environmental-unfriendliness several crude extracts from various plants have been tested in management of agriculturally economical pests and pathogens. However, many extracts obtained from plants with bio-pesticidal properties have been documented as being highly phytotoxic. Hence, the proposed study would be carried out to investigate allelopathic effect of *C. cactiformis* plant extract, based on the presence of allelochemicals on seed germination and seedling growth of lettuce prior to its adoption as alternative bio-pesticide.

1.3 Motivation

Extract of *C. cactiformis* when applied as post-emergent bionematicide reduced *Meloidogyne incognita* nematode juveniles and egg number/g roots (Khosa, 2013*)*. However, several studies have shown that extracts of various plants contain natural occurring allelochemicals which are usually highly phytotoxic. Some of the phytotoxicity noted were an inhibition of seed germination, seed killing, and necrosis of roots and inhibition of root growth. Thus, it is important to evaluate allelopathic effect of *C. cactiformis* plant extract based on the presence of allelochemicals.

1.4 Aim

The aim of the study was to investigate the allelopathic effect of *C. cactiformis* plant extract on seed germination and seedling growth of lettuce based on the presence of allelochemicals.

1.5 Objectives

1. To evaluate whether extract of *C. cactiformis* plant with determined allelochemicals would have allelopathic effect on seed germination of lettuce.

2. To evaluate whether extract of *C. cactiformis* plant with determined allelochemicals would have allelopathic effect on seedling growth of lettuce.

1.6 Hypotheses

1. Extract of *C. cactiformis* with determined allelochemicals had no allelopathic effect on seed germination of lettuce.

2. Extract of *C. cactiformis* with determined allelochemicals had no allelopathic effect on seedling growth of lettuce.

1.7 Format of the mini-dissertation

Subsequent to this General Introduction, literature on the research problem was reviewed (Chapter 2). Then, the subsequent chapters addressed above hypotheses in Chapters 3 - 4, followed by Chapter 5, which provided a summary of the study, conclusion and the recommended future research.

CHAPTER 2
LITERATURE REVIEW

This review focuses on (i) what has already been written on the research problem, including the findings and/or contradictions; (ii) existing gaps on the research problem and (iii) explanation of how the existing gaps would be addressed.

2.1 Work done on the research problem

2.1.1 Allelopathic activities of plant with bio-pesticidal potential

Various plants with bio-pesticidal properties have also been found to possess detrimental effects on growth of associated and next-season crops (Khanh *et al*., 2005). Extract of neem (*Azadirachta indica*), has been widely used as bio-insecticide on various economical important pests (Xuan *et al.,* 2004). However, Ashrafi *et al*. (2008) reported that extract of neem leaves significantly inhibited the germination percentage and seedling growth of target plant species. Inhibited germination was also observed in bean (*Phaseolus vulgaris*), tomato (*Lycopersicon esculentum*) and pepper (*Capsicum annuum*) when using extract of black nightshade (*Solanum nigrum)*, lambsquarters (*Chenopodium album)* and chamomile (*Matricaria chamomilla)* weeds (Kadioglu *et al.,* 2005). Extracts from roots and leaves of catmint (*Nepeta meyeri)* inhibited seedling growth of barley (*Hordeum vulgare*) and sunflower (*Helianthus annuus*) by 87% and 67%, respectively (Mutlu and Atici, 2009). Soil amended with ryegrass extracts inhibited emergence of Korean lawn grass (*Zoysia japonica)* when used as an organic bio-pesticide (Zuk and Fry, 2006). Recently, various monocotyledonous and dicotyledonous crop seeds showed allelopathic responses when bio-assayed with extract of *C. myriocarpus* fruit, when used as a pre-emergent bio-nematicide (Mafeo, 2012).

2.1.2 Plant-plant allelopathic interactions

Plant-plant allelopathic interactions are primarily based on the synthesis and release of secondary metabolites (allelochemicals) from one plant (donor) to another plant

(receiver) that might initiate a wide array of biochemical reactions and induce several biological changes (El-Khatib and Abd-Elaah, 1998). In nature, many plant species grow together and interact with each other by inhibiting or stimulating the growth and development through allelopathic interactions.

Biological activities of receiver plants in response to allelochemicals are known to be concentration dependent. These responses are characteristically, stimulation or attraction at low concentrations and inhibition or repellence as the concentration increases (Lovett *et al.,* 1989). When receiver plants are exposed to higher concentrations of allelochemicals, their growth and development are adversely affected. These effects include inhibition or retardation of seed germination, reduced root and shoot growth, reduced dry weight accumulation, and lowered reproductive capacity (Suman *et al*., 2002).

The allelochemicals naturally occurring in various forms are divided into different categories according to their different structures and properties (Putnam, 1985). These allelochemicals include water-soluble organic acids, straight-chain alcohols, aliphatic aldehydes, and ketones; simple lactones; long-chain fatty acids and polyacetylenes; quinines (benzoquinone, anthraquinone and complex quinines), phenolics; cinnamic acid and its derivatives; coumarins; flavonoids; tannins, steroids and terpenoids (sesquiterpene lactones, diterpenes, and triterpenoids). They are found in various plant organs such as roots, rhizomes, stems, barks, leaves, flowers, fruits (Rice, 1989). The allelochemicals from donor plants are released into the environment through different processes such as the volatilization, root exudation, leaching and decomposition of plant residues (Suman *et al.,* 2002). Leaves might be the most consistent source, while roots are considered to contain fewer and less potent allelochemicals (Kobayashi, 2004).

Naturally, the impact of allelochemical is centralized on a fine-tuned regulatory process in which these bio-chemicals act together (Cheng, 1992). Allelochemicals effect from donor plants might be either direct or indirect to receiver plants (Tanveer *et al.,* 2010). Effects through the alternation of soil properties, nutritional status and an altered population or activity of micro-organisms and nematodes represent in the indirect action. While, direct action involves the bio-chemical and physiological

effects of allelochemical on various important processes of receiver plant growth and metabolism (Chandra and Mali, 2012).

After release, allelochemicals cause both inhibitory and stimulatory effects (Mizutani, 1999). Various factors like concentration, flux rate, age, metabolic state and environmental conditions determine their toxicity (Gallet and Pellissier, 1997). Their amount and production varies in quality and quantity with age, cultivars, plant organ, and time of the year (Cambier *et al.,* 2000). The receiver plants show varied types of responses to the allelochemicals released from donor plants. These responses include stimulation or inhibition of seed germination and growth and the responses are concentration dependent.

2.1.3 Assessing allelopathic activities of plants

In assessing allelopathic potential of plants, bioassays form an integral part. Bioassays are necessary and useful aids in the study of allelopathy. Various laboratory bioassays have successfully been applied, each developed and adapted to meet specific requirements regarding donor species, target species, test medium, developmental stage, or response parameters (Wu *et al.,* 2001). Bioassays are not standardized, however; laboratory bioassay using aqueous crude extracts is the first step used to investigate the possible allelopathic potential of a plant (Foy, 1999). Many bioassays have been designed to identify the role of allelopathy in plant-plant allelopathic interactions. These bioassays can basically be categorized as aqueous extract screening, seedling screening, field screening and chemical screening (Dilday *et al*., 1994). An essential need in studying crop allelopathy is stimulation for natural release of allelochemicals so that chemical interference from living donor plants on living receiver plants can be measured (Olofsdotter *et al.,* 1995).

The inhibition or stimulation of seed germination has been the most widely used bioassay for the determination of allelopathic activity (Salisbury and Ross, 1992). Seed germination begins with imbibition of water and ends with the protrusion of the radicle through the testa. Radicle elongation is by cell extension only and does not

involve cell division. The biochemical events associated with germination are not well defined and may only be preparatory for the mobilization of reserves for seedling growth. Thus, definitive conclusions of allelopathic mechanisms in seed germination bioassays are limited. But, it involves membrane alteration, resulting in loss of metabolites and the ability to establish the necessary osmotic potential for cell elongation (Koller and Hadas, 1982). Other processes, such as alteration of the phytochrome control of germination, may also be affected. Other studies found that some naturally occuring volatile compounds stimulated the dark germination of *Rumex* sp. that normally requires post-imbibitional light (French and Leather, 1979).

Seedling growth bioassays are extremely versatile but require a greater quantity of chemical than is usually available during initial isolation and identification of allelochemicals (Blum *et al.,* 1985). These bioassays usually have greater sensitivity and provide the basis for a variety of mechanism studies. In allelopathic interactions, some phytotoxic substances are released by donor plants into the environment to affect growth of receiver plants. Whereas, in competitive interactions, a growth resource is removed from the environment by one plant so that the growth resources available to other plants are reduced (Wu *et al.,* 2000).

2.2 Work not yet done on the research problem

The botanical description, growth habitat and medicinal properties of *C. cactiformis* plant have been well documented by Van Wyk *et al.* (1997) and Wink and Van Wyk (2008). Recently, Khosa (2013) screened extract of *C. cactiformis* as bio-nematicide against *Meloidogyne incognita* race 2 using tomato as the test plant. Results in both the microplot and field experiments showed that the materials reduced nematode juveniles by 98-100% and eggs 90-98%, respectively. However the use of *C. cactiformis* plant extract on germination and seedling growth as bio-nematicide on lettuce seeds has not been investigated.

2.3 Addressing the identified gaps

Plant species with potential to be used in pathogens, pest and weed management had also shown allelopathic effect on other plants growing in their vinicity (Al-Watban

and Salama, 2012; Mafeo, 2012). Allelopathic effects are very common for many plant species and can be observed at any level of biological organization (Al-Rabiah, 2012). Hence, it is important to investigate whether extract of *C. cactiformis* plant when applied as pre-emergent bio-nematicide would not affect other plants growing in as test crops. In establishing *C. cactiformis* extract allelopathic activities, seed germination and seedling growth bioassays would be used. The two bioassays have been the most widely used test tools to determine allelopathic activities of a particular plant species against other plants.

CHAPTER 3
RESEARCH METHODOLOGY

3.1 Experimental sites, design and treatments

3.1.1 Seed germination bioassay

Leaf and stem parts of *C. cactiformis* plant were collected from selected traditional healers from the different localities in Mopani (23°19′S, 30°43′E) and Vhembe (22°56′S, 30°28′E) Districts in Limpopo Province, South Africa. The bioassay was conducted in the Department of Plant Production and Soil Science laboratory of University of Limpopo (23^{o}88′06”S, 29^{o}73′39”E).

Four treatments namely, 0, 0.5, 1 and 1.25 mg/mℓ of acetone, methanol, ethanol and distilled water extracts of *C. cactiformis* plant were arranged in a completely randomised design with four replicates. Lettuce (*Lactuca sativa L*.) seeds were purchased from local market and were chosen as test plants for the bioassay due to their ability to grow faster and sensitivity to phytotoxic chemicals (Salam and Kato-Noguchi, 2010).

3.1.2 Seedling growth bioassay

The seedling growth bioassay study using extract of *C. cactiformis* plant were conducted at Horticultural Unit of the University of Limpopo (23º53’10”S, 29º44’15”E) under greenhouse conditions. Six treatments *viz*. 0, 0.5, 1.0, 1.25, 2.0 and 2.25 g/pot of *C. cactiformis* plant extract, were laid-out in a complete randomised design, with four replications.

3.1.3 Phytochemical analysis

Phytochemical analysis was conducted in the Department of Biochemistry laboratory, University of Limpopo (23°88′46”S, 29°73′829”E). Thin layer chromatography (TLC) with aluminium baked silica-gel coated plates (Sigma-Aldrih, SA) was used to determine the presence of coumarins, phenolic compound, phytosterols and separation of flavonoids glycoside in the extract (Appendix 3.1)

whereas, saponins, terpenoids, flavonoids, tannins, alkaloids and cardiac glycoside were determined by chemical tests (Appendix 3.2).

3.2 Plant sampling and extract preparation

Fresh *C. cactiformis* plant materials were chopped into pieces after collection, dried at 65^{o}C in air-forced ovens to minimise the loss of volatile phytochemicals according to Makkar (1999). Dried materials were ground in a Wiley mill through 1mm-mesh sieves and warring blender. Prior to use, the milled plant materials were stored in appropriately marked and sealed plastic bags at room temperature. The plastic bags were kept away from direct sunlight and in an area where temperature fluctuations were minimal. The solutions used for treatment were extracted by mixing 100g of milled plant material into 1ℓ of acetone, distilled water, ethanol and methanol and shook for 24-hours at 25^{o}C by LABCON shaker (Model: 3100U) at 200rpm. The solutions were sieved using 210µm, 106µm, 75µm and 45µm mash-sized sieves and then filtered through cotton wool to remove the fibre debris. Later all the extracts were concentrated using rotary flash evaporator and preserved at 4^{o}C in air tight bottle until further use.

3.3 Germination and seedling growth bioassays

The dried extracts of about 3.5g of acetone, methanol, ethanol and distilled water fraction were re-dissolved in 10mℓ of acetone, methanol, ethanol and distilled water, respectively. About 5mℓ of each of the solution was added to a sheet of filter paper (Whatman; no 1) in a 9cm petri-dish and dried to allow for total evaporation of dissolving solvents. Thereafter, the filter paper were moistened with 10mℓ of 3mM phosphate buffer (pH 7.0) containing 0.05% Tween 20 (polyoxyetylenesorbitan monolaurate, Sigma). One hundred and sixty lettuce seeds were surface sterilized with 10:1 water/bleach (3.5% w/v NaClO) solution and ten seeds were seeded on the Whatman filter paper in each sterilized 9cm petri-dishes. The petri-dishes were sealed and placed in a Fitotron (Model: SGC 120) growth chamber (Appendix 3.3). It was regulated at 25^{o}C temperature and lights during the day and 12^{o}C temperature at night for a period of seven days with relative humidity of 75%. A 2.5mℓ solution of each treatment was re-applied every other day.

For seedling growth twenty four 10cm diameter plastic pots were placed on the greenhouse bench (Appendix 3.4).The pots were filled with growing mixture, comprising 2:1 v/v steam pasteurised sand and Hygromix seedling growing medium (Hygrotech, South Africa). Two seeds per hole were planted at commercially prescribed depths of about 0.5cm (Hygrotech Planting Guide, 2009). And ground *C. cactiformis* plant extract was applied as pre-emergent bio-nematicide in separate holes around the seeds at the same depths and covered with the growing mixture. Pots were irrigated with 100mℓ to field capacity prior to planting and then with 50mℓ tap water every other day. Plants were thinned to one per pot soon after germination to alleviate competition.

3.4 Phytochemical analysis

3.4.1 Phytochemical analysis performed using thin layer chromatography (TLC)

The TLC was performed on coated 20×20cm and 0.25mm thick plates. Stock solution of 10 mg/mℓ was prepared by dissolving 10mg of the dried samples of the extracts in 1mℓ of acetone. Following spotting (10µℓ), the plates were eluted in six solvent systems with varying polarity namely, ethyl acetate: chloroform: water (ECW 5:3:1), methanol: water (MW 3:6), hexane: ethyl acetate (HE 5:2) and chloroform: ethyl acetate (CE 5:7) under saturated conditions. After developing TLC, the solvent front was drawn and the plates were allowed to dry in a fume hood before visualizing. After performing TLC for all the extract, the R_f values for different spots were determine as described by Chakraborty *et al*. (2010). The fluorescing bands were also visualised under ultraviolet light at 254nm and 365nm.

3.4.2 Phytochemical analysis performed using Chemical test

1. Terpenoids (Salkowski Test)

About 0.5g of each extract was added to 2mℓ of chloroform. Concentrated H_2S0_4 (3 mℓ) was carefully added to form a layer. A reddish brown colouration of the interface indicates the presence of terpenoids.

2. Flavonoids

Diluted ammonia (5mℓ) was added to a portion of an aqueous filtrate extract. Concentrated sulphuric acid (1mℓ) was also added. A yellow colouration that disappears on standing indicates the presence of flavonoids.

3. Saponins

For the test of saponins, 0.5g of extract was added to 5mℓ of distilled water in a test tube. Test solution was shaken vigorously and observed for a stable persistent froth. The formation of emulsion indicates the presence of saponins.

4. Tannins

About 0.5g of the extract was boiled in 10mℓ of water in a test tube and then filtered. A few drops of 0.1% ferric chloride was added and observed for brownish green or blue black colouration.

5. Alkaloids (Dragendorff's test)

Solvent free extract, 50mg was stirred with 2mℓ of diluted hydrochloric acid and filtered. To a few mℓ of filtrate, 1 or 2mℓ of dragendorff's reagent was added by the side of the test tube. A prominent yellow indicates positive test.

6. Cardiac glycosides (Keller-Killiani Test)

About 0.5g of extract diluted to 5mℓ in water, 2mℓ of glacial acetic acid containing one drop of ferric chloride solution was added. This was underplayed with 1mℓ of concentrated sulphuric acid. A brown ring at the interface indicates the presence of deoxysugar characteristic of cardenolides. A violet ring might appear below the brown ring.

3.5 Data collection and analysis

Germination was determined by counting the number of germinated seeds at 24-h intervals over a period of 6 days and expressed as mean germination percentage. Radicle and hypocotyl length (cm) were measured using a ruler 8 days after seeding by measuring 16 representative seedlings. Four weeks after planting, successful seedling emergence was recorded; seedling length (cm) was measured using a ruler. Fresh and dry biomass was measured using NHB compact balance electronic scale (Model: HCB 1002) and chlorophyll content of the leaves using Opti-sciences chlorophyll meter (Model: CCM-200 plus). Data for both seed germination and seedling growth variables were subjected to analysis of variance (ANOVA) with the Statistix 9.0 (Statistix Institude Inc, 1985 to 2009). Mean separation at $P \leq 0.01$ was achieved using Least Significant Difference test (LSD) and Duncan's multiple-range test at probability level of 0.05.

CHAPTER 4
RESULTS AND DISCUSSION

4.1 Results

4.1.1 Seed germination bioassay

In all variables measured, treatments were significantly different at ($P \leq 0.01$) for acetone, ethanol, distilled water and methanol of *C. cactiformis* plant extract (Table 4.1). Partitioning of the sum of square for acetone, distilled water, methanol and ethanol extracts indicated that the total treatment variation in seed germination was influenced by 77%, 91%, 92% and 99%, respectively. Similarly, the effect of extracting solvents on hypocotyl length was influenced depending on the type of solvent used with acetone, distilled water, methanol and ethanol having an effect of 75%, 87%, 99% and 99%, respectively. Distilled water extracts had a reduction of 19 % on radicle length when compared to the other solvent types with methanol extracts having the least effect. In general, responses by test plant to different solvent extracts were concentration dependant.

Comparisons of mean seed germination percentage as influenced by different solvent extracts showed a variation in 2 groups where means were significantly different from one another. Mean germination percentage for control was higher as compared to other concentrations of each extract types (Figure 4.1). Similarly, hypocotyl length and radicle length had 2 groups in which means were not significantly different from one another. Also, in all extract types hypocotyl and radicle length for control were different as compared to other levels of each extract type (Figure 4.2-4.3).

4.1.2 Seedling growth bioassay

Extract of *C. cactiformis* plant did not significantly differ at ($P \leq 0.05$) for all lettuce growth variables measured. Partitioning of the sum of square indicated that the degree of treatment explained 18%, 27%, 28%, 30% and 31% of the treatment

variation in seedling emergence, seedling height, chlorophyll content, fresh biomass and dry biomass weight of lettuce as test plant, respectively (Table 4.2).

There were no significant pairwise differences among the means for seedling emergence, seedling height and chlorophyll content (Figure 4.4-4.6). Whereas, for fresh and dry biomass weights there were two 2 groups in which means were not significantly different from one another (Figure 4.7-4.8).

4.1.3 Phytochemical analysis

The phytochemical analysis results of *C. cactiformis* plant extract revealed some different in the allelochemicals of the extract types tested. In the acetone, methanol and ethanol extract of *C. cactiformis* plant, saponins, terpenoids, flavonoids, tannins, alkaloids, coumarins, phytosterols were present, with phenolic compounds and cardiac glycoside being absent. However, in the distilled water extract terpenoids, flavonoids, tannins, alkaloids, coumarins and phenolic compounds were present, with the saponins and cardiac glycoside being absent (Table 4.3).

Table 4.1 Partitioning of the treatment sum of square derived from the analysis of variance (ANOVA) for extract of *C. cactiformis* plant on lettuce seed germination %, hypocotyl length and radicle length (n=4).

		EXTRACT TYPES											
		Acetone extract						**Ethanol extract**					
SV	**DF**	**Germ (%)**		**Hypocotyl (cm)**		**Radicle (cm)**		**Germ (%)**		**Hypocotyl (cm)**		**Radicle (cm)**	
		SS	%	SS	%	SS	%	SS	%	SS	%	SS	%
REP (A)	3	868.75	4.1^{ns}	1.08	3.6^{ns}	0.25	5.8^{ns}	25.0	0.19^{ns}	0.009	0.05^{ns}	0.07	0.99^{ns}
TRT (B)	3	16068.7	77^{***}	22.31	75^{***}	2.90	67^{***}	12675	99^{***}	17.31	99^{***}	6.77	96^{***}
A*B	9	4006.25		6.28		1.16		75.0		0.02		0.22	
TOTAL	15	20943.7		29.69		4.31		12775		17.35		7.06	
		Distilled water extract						**Methanol extract**					
SV	**DF**	**Germ (%)**		**Hypocotyl (cm)**		**Radicle (cm)**		**Germ (%)**		**Hypocotyl (cm)**		**Radicle (cm)**	
		SS	%	SS	%	SS	%	SS	%	SS	%	SS	%
REP (A)	3	300.0	3.1^{ns}	1.050	5.4^{ns}	0.010	0.54^{ns}	168.7	1.8^{ns}	0.016	0.16^{ns}	0.04	0.86^{ns}
TRT (B)	3	8650	91^{***}	16.74	87^{***}	0.369	19^{***}	8268	92^{***}	9.45	99^{***}	5.16	97^{***}
A* B	9	550		1.44		0.092		506.2		0.048		0.13	
TOTAL	15	9500		19.23		1.969		8943		9.489		5.35	

SV=Source of variation, DF=Degree of freedom, SS= Sum of square, ^{ns} Not significant at ($P \leq 0.01$), *** = Highly significant at ($P \leq 0.01$).

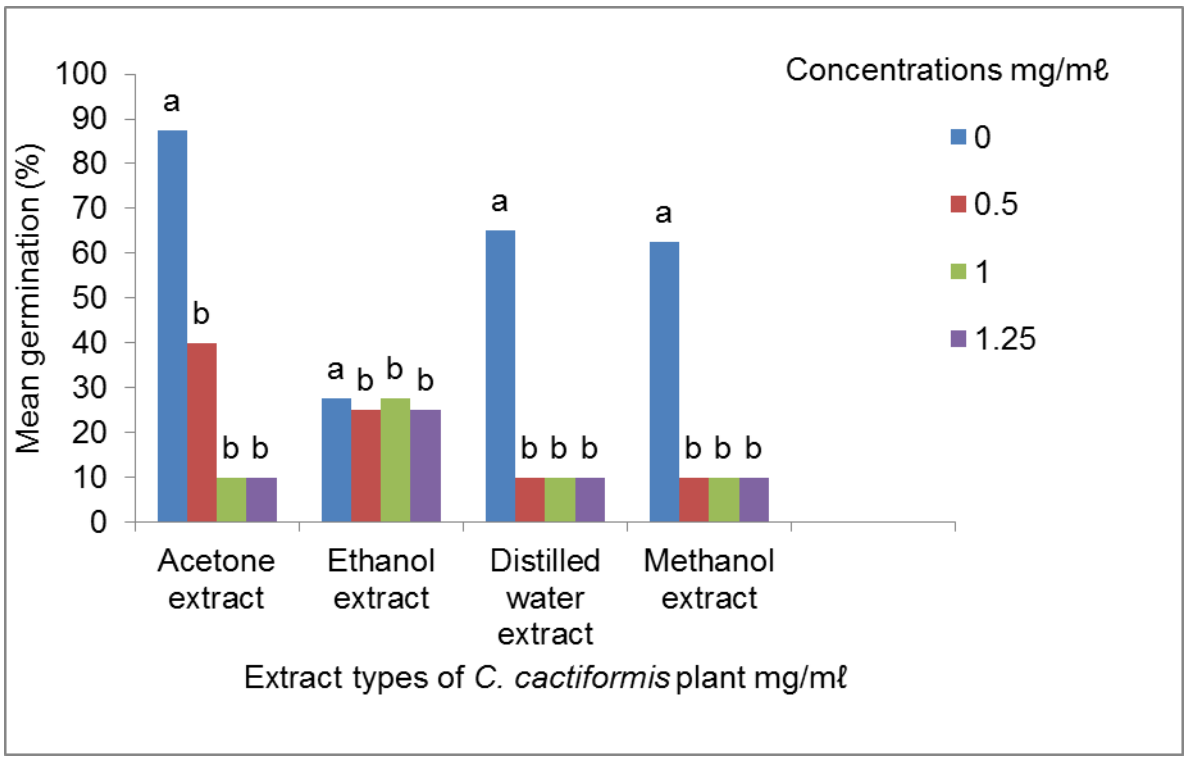


Figure 4.1 Mean germination % treated with different extract types of *C. cactiformis* plant (n=4).

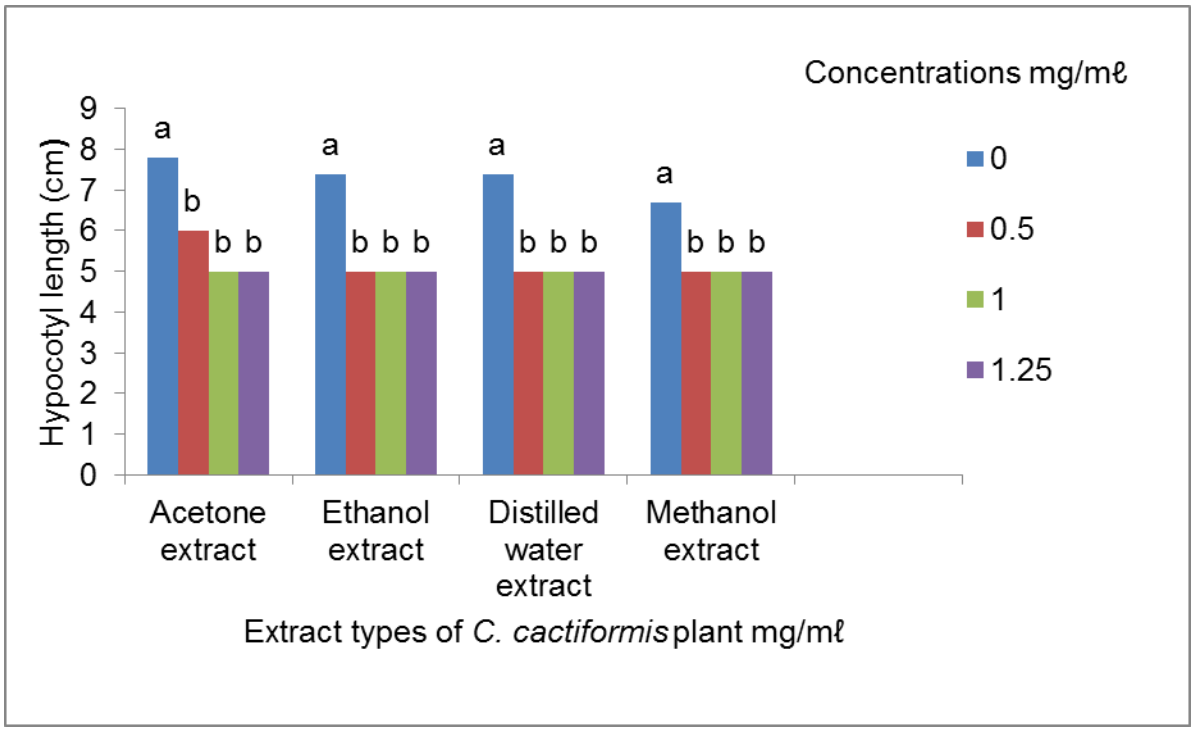


Figure 4.2 Mean comparison for hypocotyl length treated with different extract types of *C. cactiformis* plant (n=4).

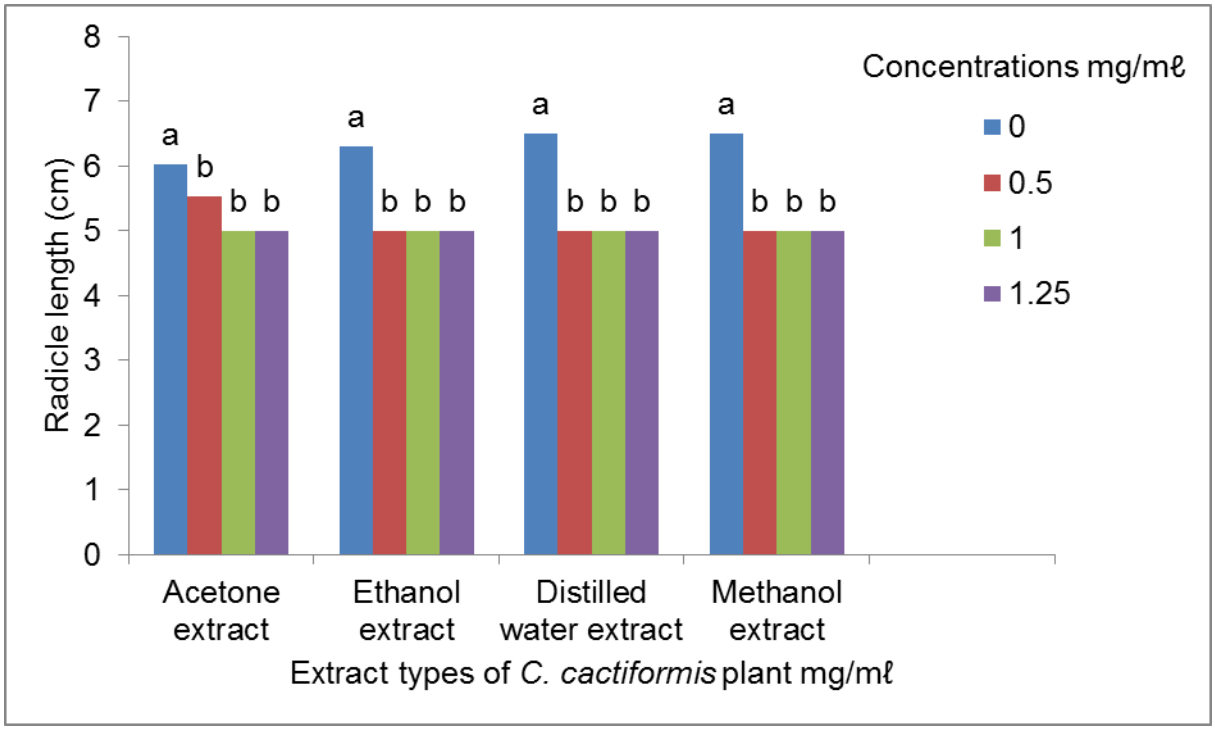


Figure 4.3 Mean comparison for radicle length treated with different extract types of *C. cactiformis* plant (n=4).

Table 4.2 Partitioning of the treatment sum of square derived from the analysis of variance (ANOVA) for extract of *C. cactiformis* plant on seedling growth of lettuce (n=4).

SV	**DF**	**[1]SE**		**[2]SH**		**[3]CC**		**[4]FBW**		**[5]DBW**	
		SS	**(%)**	**SS**	**(%)**	**SS**	**(%)**	**SS**	**(%)**	**SS**	**(%)**
REP (A)	3	0.17	9.28^{ns}	69.84	12.44^{ns}	8.23	9.63^{ns}	1.004	11.88^{ns}	0.001	5.26^{ns}
TRT (B)	5	0.33	18^{ns}	154.80	27^{ns}	24.36	29^{ns}	2.551	30^{ns}	0.006	32^{ns}
A*B	18	1.33		336.88		52.80		4.899		0.012	
TOTAL	23	1.83		561		85.38		8.454		0.019	

SV= Source of variation, DF= Degree of freedom, [1]SE= Seed emergence, [2]SH=Seedling height, [3]CC=Chlorophyll content, [4]FBW=Fresh biomass weight, [5]DBW=Dry biomass weight, [ns]Not significant at ($P \leq 0.05$).

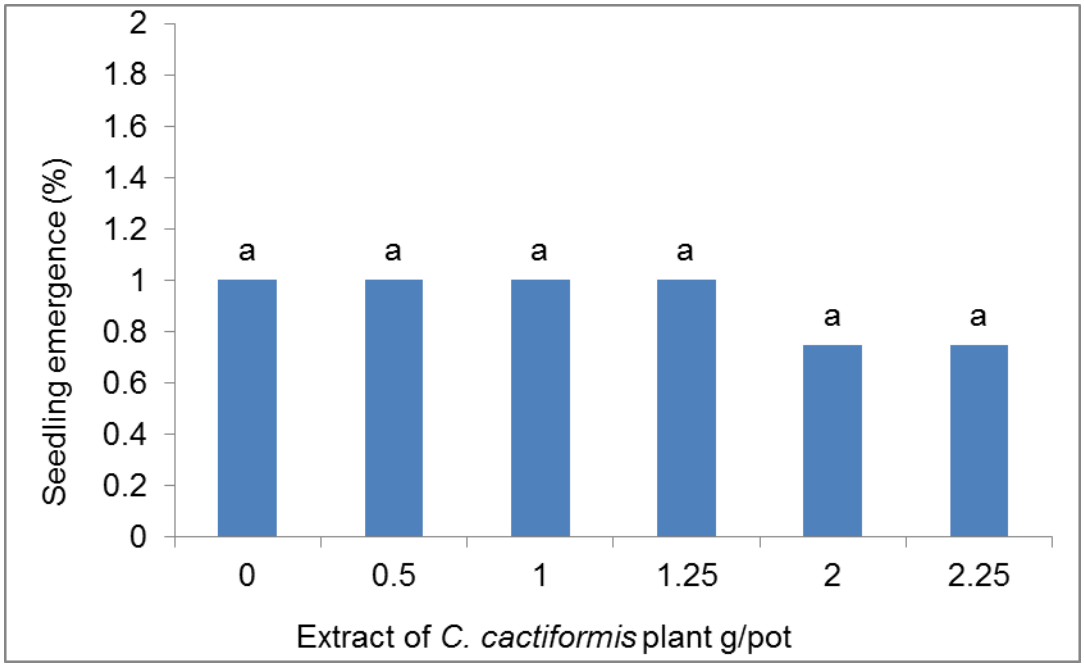


Figure 4.4 Mean comparison for seedling emergence as influence by extract of *C. cactiformis* plant (n=4).

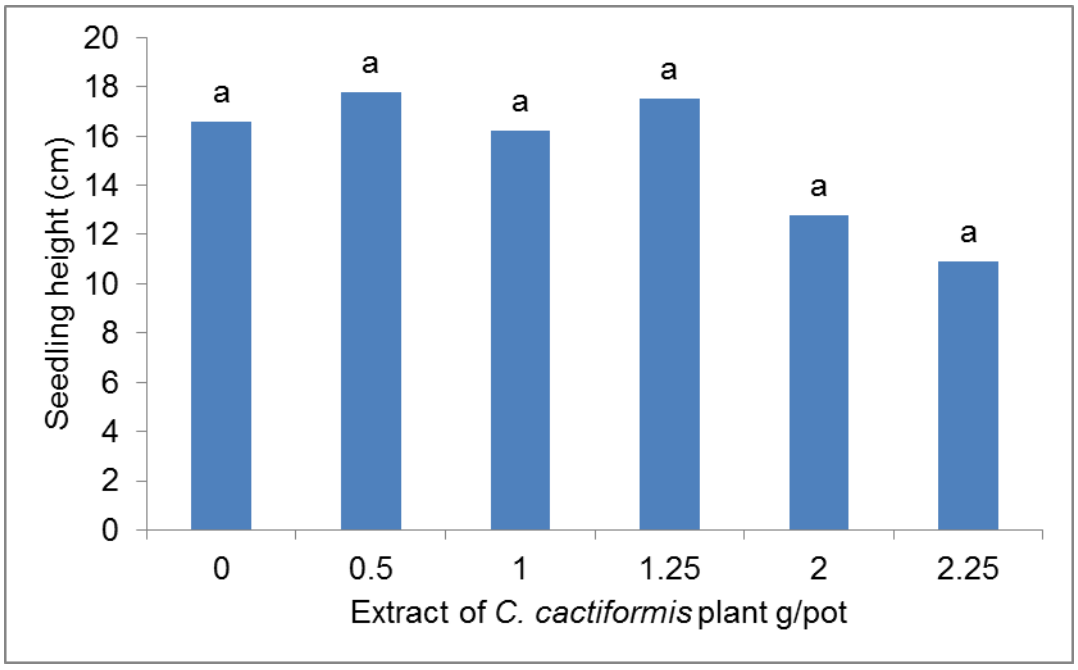


Figure 4.5 Mean comparison for seedling height as influence by extract of *C. cactiformis* plant (n=4).

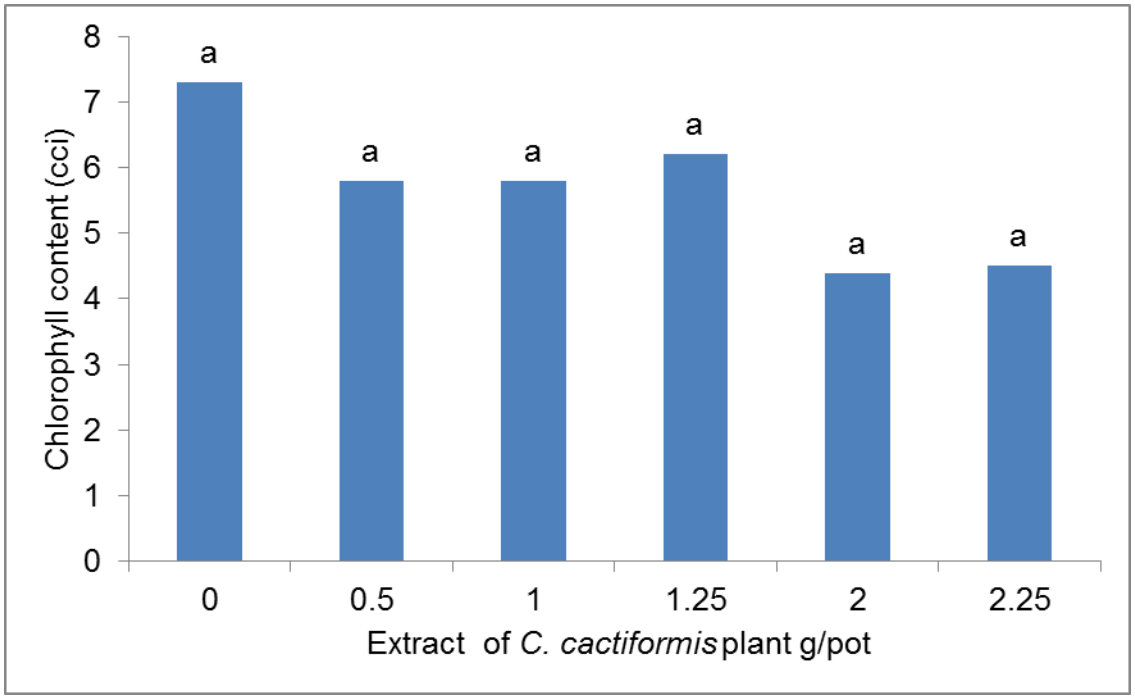


Figure 4.6 Mean comparison for chlorophyll content as influence by extract of *C. cactiformis* plant (n=4).

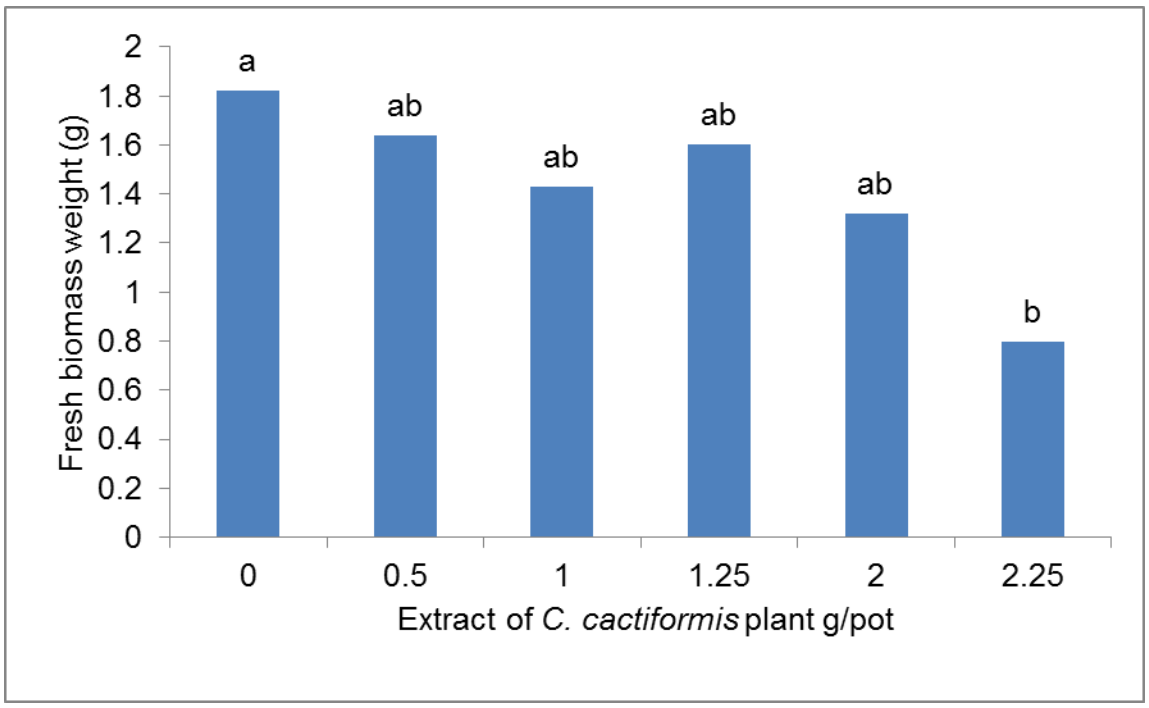


Figure 4.7 Mean comparison for fresh biomass weight as influence by extract of *C. cactiformis* plant (n=4).

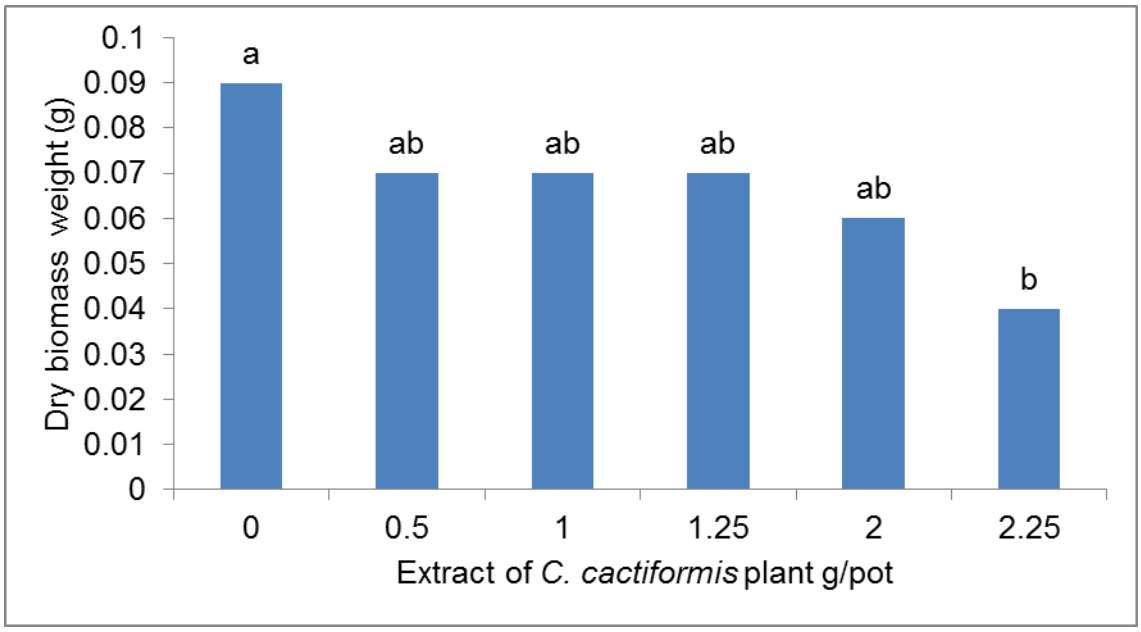


Figure 4.8 Mean comparison for dry biomass weight as influence by extract of *C. cactiformis* plant (n=4).

Table 4.3 Phytochemical analysis of *C. cactifomis* plant extracted by different solvents.

	EXTRACT TYPES			
Phytochemicals	**Acetone extract**	**Methanol extract**	**Ethanol extract**	**Distilled water extract**
1. Saponins	+	+	+	-
2. Terpenoids	+	+	+	+
3. Flavonoids	+	+	+	+
4. Tannins	+	+	+	+
5. Alkaloids	+	+	+	+
6. Cardiac glycoside	-	-	-	-
7. Coumarins	+	+	+	+
8. Phenolic compounds	-	-	-	+
9. Phytosterols	+	+	+	-
10. Separation of flavonoid aglycone	+	+	+	-

Note: (+) = Present (-) = absent

4.2 Discussion

Germination bioassay together with phytochemical analysis has been widely used to determine allelopathic activities of various plant extracts and their allelochemicals (Hoagland and Williams, 2004). In this study, different solvents extract of *C. cactiformis* plant showed different allelopathic effect on all measured lettuce variables on germination bioassay. This observation agreed with previous works which showed that acetone extract had the most stimulatory effect as compared to butanol and ethanol extract on lettuce and tomato crops (Hassan and Ghareib, 2009). The obtained data indicated that acetone extract had stimulatory effect on the mean germination of lettuce at lower concentration. The stimulatory effect could be attributed to the possible synergistic effect between the tested allelochemicals as mentioned by Gerig and Blum (1991).

Similar responses to the material were observed on hypocotyl length and radicle length. This result showed that radicle length was more sensitive to allelochemicals when compared with hypocotyl length. The inhibition of ethanol extract on the variables hypocotyl length and mean germination percentage was greater than on radicle length of the test plant. The current findings agreed with that of Stachon and Zimdahl (1980) who found the ethanol extracts of Canada thistle (*Cirsium arvense)*) to be more inhibitory to cucumber (*Cucumis sativis*) radicle than to hypocotyl. The differential response by radicle could be explained by its sensitivity to allelochemicals than any tissue of developing seedling.

The seed germination variables were inhibited to a varying degree by methanol and distilled water extract. Differential effect of the methanol and distilled water extract on measured germination variables of lettuce might be due to the presence of different compounds in each extract solvent. In this study, high concentration of extract type showed significant inhibition effect on seeds germination and seed bursting was also observed. Results agreed with the finding of Ladwig *et al.* (2012) where several Vitaceae species shown allelopathic effect when bio-assayed on common cultivated crops. When bio-assayed on radish seeds, extracts of *Parthenocissus quinquefolia* plant and *Vitis vulpine* inhibited germination continually with increasing solvent extracts concentration (Batish *et al.,* 2007).

Contrarily, seedling growth results showed that ground *C. cactiformis* plant extract had neither inhibitory nor stimulatory effect on seedling height, chlorophyll content, fresh biomass and dry biomass weight of lettuce. In other words, there was no significant relationship observed between extracts concentrations and measured variables of the target crop. Similarly, root extract of *Aloe ferox* showed no significant effect on the germination of carrot at all concentrations (Arowosegbe *et al.,* 2012). Physiologically, concentration-dependent growth patterns suggest that there is, depending on the concentration, the constant growth phase, the stimulation growth phase, followed by peak growth phase and then the inhibition growth phase (Salisbury and Ross, 1992). According to this biological growth system, responses by all measured lettuce variable were still at constant growth phase, suggesting that the extract of *C. cactiformis* plant applied was not sufficient to influence lettuce seedling growth. These results, explained why the use of this material had no inhibitory or stimulatory effects on tomato crop when applied as bio-nematicide (Khosa, 2013).

Phytochemical analysis of *C. cactiformis* plant materials revealed the presence of various allelochemicals in different extracts type. The detected allelochemicals in this study have been associated with allelopathy. Among the allelochemicals that have been implicated in inhibiting seed germination and seedling growth includes benzoquinones, coumarins, flavonoids, terpenoids, lactones, alkaloids and phenolic compound (Marcias *et al*., 2002). The presence of more than one allelochemical in a solvent extract leads to either inhibitory or stimulatory effect to a test plant as their actions are usually synergistic (Al-Watban and Salama, 2012).

CHAPTER 5
SUMMARY, CONCLUSION AND FUTURE RESEARCH

The importance of *C. cactiformis* plant extract as an alternative bio-nematicide in nematode suppression is well-documented (Khosa, 2013). However, its allelopathic activities on other plants would need to be evaluated prior to its adoption as commercial bio-nematicide. Hence, its phytotoxicity to other plants was evaluated using seed germination and seedling growth bioassays as the basic allelopathic determination tools. Furthermore, allelochemicals (phytochemicals) that might be responsible for *C. cactiformis* plant allelopathic activities in the two bioassays were also screened.

Extract of *C. cactiformis* plant confirmed that irrespective of the solvent used, the extracts had allelopathic effect on germination of lettuce. The inhibitory and stimulatory effect was the function of the extract concentration in all extract types. The greatest inhibitory effect was observed under the application of high extract concentration. Thus, further studies could be necessary to elucidate allelopathic activities of this material on other agricultural crops with nematode infection problem.

In seedling growth bioassay, the material showed no allelopathic effect towards lettuce as the test plant. This result explained why the use of the plant material as a bio-nematicide at 2g\pot\plant had no phytotoxicity effect on tested crop (Khosa, 2013). However, determination of Mean Dosage Response for Stimulation (Mafeo, 2012) for the material when used as post-planting bio-pesticide still needs to be determined for this material.

On the phytochemical screening study, results showed the presence of different allelochemicals in different extract types. This observation explained the similarities in obtained synergistic allelopathic pattern responses by different solvent extract types on seed germination bioassay. However, validation of the screened allelochemicals results in this study, is recommended.

REFERENCES

ABU-ROMMAN, S., SHATNAWI, M., and R.S. SHIBLI. 2010. Allelopathic effects of Spurge (*Euphobia hieroslymitana*) on Wheat (*Triticum durum*). *Journal of Agricultural Environmental Science* 7: 298-302.

AL-RABIAH, H.K. 2012. Allelpathic effects of *Citrullus colocynthis* extracts on the germination and growth of some plants. MSc. Thesis, Bot. Dept., Fac. Sci., King Saud Uni., Saudi Arabia.

AL-WATBAN, A., and M.H. SALAMA. 2012. Physiological effects of allelopathic activity of *Artemisia monosperma* on common bean (*Phaseolus vulgaris* L.). *Journal of Plant Science* 3: 158-163.

ANAYA, A.L. 1999. Allelopathy as a tool in the management of biotic resources in agro ecosystems. *Plant Science* 18: 697-739.

AROWOSEGBE, S., WINTOLA, O.A., and A.J. AFOLAYAN. 2012. Phytochemical constituents and allelopathic effect of *Aloe ferox* Mill. root extract on tomato. *Journal of Medicinal Plants Research* 6: 2094-2099.

ASHRAFI, Y. Z., RAHNAVARD, A., and S. SADEGHI. 2008. Study of the allelopathic potential of extracts of *Azadirachta indica. Journal of Biological Sciences* 8: 56-61.

AYENI, A.O., LORDBANJOU, D.T., and B.A. MAJEK. 1997. *Tithonia diversifolia* (Mexican sunflower) in South Western Nigeria; occurrence and growth habit. *Weed Research* 37: 443-449.

BATISH, D.R., LAVANYA, K., SINGH, H.P., and R.K. KOHLI. 2007. Root mediated allelopathic interference of Nettle-leaved Goosefoot *(Chenopodium murale*) on wheat (*Triticum aestivum*). *Journal of Agronomy and Crop Science* 193: 37-44.

BLUM, U., DALTON, B.R., and J.R. SHANN. 1985. Effects of ferulic and p-coumaric acids in nutrient culture on cucumber leaf expansion as influenced by pH. *Journal of Chemical Ecology* 11: 1567-1582.

CAMBIER, V., HANCE, T., and E. HOFFMANN. 2000. Variation of dimboa and related compounds content in relation to the age and plant organ in maize, *Phytochemistry* 53: 223-229.

CHAKRABORTY, B.**,** NANDY, S., and R. DATTA. 2010. Phytochemical and pharmacognostical studies of the leaves of *Alangium lamarkii*. *International Journal of Biomedical Research* 3: 196-200.

CHANDRA, J., and M.C. MALI. 2012. Allelopathic effect of *Acacia tortilis* on germination and seedling growth of *Prosopis chilensis*. *Life Science Leaf* 2: 53-57.

CHENG, H.H. 1992. A conceptual framework for assessing allelochemicals in the soil environment. In: S.J.H Rizvi and V. Rizvi (ed.). Allelopathy: Basic and applied aspects. Chapmann and Hall Publishers, Pp. 21-29.

CHITWOOD, D.J. 2002.Phytochemical based strategies for nematode control. *Annual Review Phytopathology* 40: 221-49.

DUKE, S.O., DAYAN, F.E., ROMAGNI, J.G., and A.M. RIMANDO. 2000. Natural products as sources of herbicides: current status and future trends. *Weed Research* 40: 99-111.

DILDAY, R.H., LIN, J., and W. YAN. 1994. Identification of allelopathy in the USDA-ARS rice germplasm collection. *Australia. Journal of Experiment for Agriculture* 34: 907-910.

FOY, C.L. 1999. How to make bioassay for allelopathy more relevant to field condition with particular reference to cropland weeds.In: Principle and Practices in Plant Ecology: Allelochemicals Interactions (Eds.: Inderjit, K.M.M. Dakshini and L.F Chester). CRC Press USA, Pp. 25-33.

FRENCH, R.C., and R.G. LEATHER.1979. Screening of non-anal and related volatile flavor compounds on the germination of 18 species of weed seed. *Journal of Agricultural and Food Chemistry* 27: 828-832.

GALLET, C.**,** and F. PELLISSIER. 1997. Phenolic compounds in natural solutions of a coniferous forest. *Journal of Chemical Ecology* 23: 2401-2412.

GERIG, T.M., and U. BLUM. 1991. Effects of mixture of four phenolic acids on leaf area expansion of cucumber seedlings grown in Portsmouth B1 soil Material. *Journal of Chemical Ecology* 17: 29-35.

HASSAN, S.M. and H.R. GHAREIB. 2009. Bioactivity of *Ulva lactuca* L. acetone extract on germination and growth of lettuce and tomato plants. *Journal of Biochemistry* 16: 3832-3838.

HOAGLAND, R.E., and R.D. WILLIAMS. 2004. Bioassay useful tool for the study of allelopathy. In: 'Allelopathy. Chemistry and mode of action of allelochemicals.' (Eds FA Macías, JCG Galindo, JMG Molinillo, HG Cutler) Pp. 315-351.

HUANG, P.M., WANG, M.C., and M.K. WANG. 1999. Catalytic transformation of phenolic compounds in the soil. In: Inderjit, et al. (ed.) Principles and practices in plant ecology: Allelochemical interactions. CRC Press, Boca Raton, FL. Pp. 287-306.

HYGROTECH PLANTING GUIDE, 2009. Hygrotech Vegetable Production/Planting Guide, Pretoria, South Africa.

INDERJIT, K., DAKSHINI, K.M.M., and C.L. FOY. 1999. Plant Ecology. CRC Press, New York, USA, Pp. 479-494.

KADIOGLU, I., YANAR, Y., and U., ASAV. 2005. Allelopathic effects of weeds extracts against germination of some plants. *Journal of Environmental Biology* 26: 169-173.

KHANH, M.A., K.B. MARWAT, G. HASSAN and Z. HUSSAIN. 2005. Bioherbicidal effects of tree extracts on seed germination and growth of crops and weed. *Pakistan. Journal of Weed Science Research* 11: 179-184.

KHOSA, M.C. 2013. An investigation into the potential of crude and partially separated material of selected non-crop plant species as control agents of root-knot nematodes (*Meloidogyne incognita*) in tomato*.* Ph.D thesis., Dept. Environmental Sci., Univ. North-West, South Africa.

KOBAYASHI, K. 2004. Factors affecting phytotoxic activity of allelochemicals in soil. *Weed Biology and management* 4: 1-7.

KOLLER, D. and A. HADAS. 1982. Water relations in the germination of seeds. In: Lange, O.L., Nobel, P.S., Osmond, C.B. and Ziegler, H. (eds), Encyclopedia of Plant Physiology. New Series, Vol. 12B. Physiological Plant Ecology II. Water Relations and Carbon Assimilation. Springer-Verlag, Berlin, Pp. 401-431.

LADWIG, L.M., MEINERS, S.J., PISULA, N.L., and K.A. LANG. 2012. Conditional allelopathic potential of temperate lianas. *Plant Ecology* 10: 1007-11258.

LEE, D.L., PRISBYLLA, M.P., CROMARTIE, T.H., DAGARTIND, D.P., HOWARD, S.W., PROVAN, W.M., M.K. ELLISM.K., FRASER, T., and L.C. MUTTER. 1997. The discovery and structural requirements of inhibitors of *p*-hydroxyphenylpyrvate dioxygenase. *Weed Science* 45: 601-609.

LOVETT, J.V., RYUNTYU, M.Y., and D.L. LIU. 1989. Allalopathy, chemical communication, and plant defence. *Journal of Chemical Ecology* 15: 1193-1201.

MAFEO, T.P. 2012. Responses of economically important crops to crude extracts of C*ucumis myriocarpus* fruits when used as a pre-emergent bio-nematicide. Ph.D thesis. Dept. Plant production and Soil Sci., Univ. Limpopo, South Africa.

MAJI, M. D., CHATTOPADHYAY, S., KUMAR, P., and B. SARATCHANDRA. 2005. In vitro screening of some plant extracts against fungal pathogens of mulberry (*Morus* spp.). *Arch. Phytopathology* 3: 157-164.

MAKKAR, H.P.S. 1999. Quantification of tannins in tree foliage. IAEA Working. Document, IAEA, Vienna: Australia.

MARCIAS, F., VARELA, R.M., TORRES, A., GALINDO, J.L.G., AND J.M.G. MOLINILO. 2002. Allelochemials from sunflowers: chemistry, bioactivity and applications. In: Inderjit and A. U. Mallik (Eds.). Chemical Ecology of Plants: Allelopathy in Aquatic and Terrestrial Ecosystems.Birkhauser Verlag, Basel, Pp. 73-87.

MASHELA, P.W. 2002. Ground wild cucumber fruits suppress numbers of *Meloidogyne incognita* on tomato in micro plots. Nematropica 32: 13-19.

MIZUTANI, J. 1999. Selected allelochemicals. *Critical Review for Plant Science* 18: 653-671.

MUTLU, S., and O. ATICI. 2009. Allelopathic effect of *Nepeta meyeri* Benth. extracts on seeds germination and seedling growth of some crop plants. *Acta Physiological Planta* 31: 89-93.

OLOFSDOTTER, M., NAVAREZ, D., and K. MOODY. 1995. Allelopathic potential in rice (*Oryza sativa* L.) germplasm. *Annual Applied Biology* 127: 543-560.

PUTNAM, A.R., 1985. Weed allelopathy. In: S.O. Duke (ed.). *Weed physiology*: Reproduction and Ecophysiology. CRC Press, Pp. 131-155.

RICE, E.L. 1989. Allelopathy-an update. *The Botanical Review* 45: 15-109.

RIMANDO, A.M., OLOFSDOTTER, M., and S.O. DUKE. 2001. Searching for rice allelochemicals. *Journal of Agronomy* 93: 16-20.

SALAM, M.A. and H. KATO-NOGUCHI. 2010. Allelopathic potential of methanol extract of Bangladesh rice seedlings. *Asian Journal of Crop Science* 2: 70-77.

SALISBURY, F.B., and C.W. ROSS. 1992. Plant physiology, 4th ed. Wadsworth, California, p. 682.

STACHON, W. J., and R. L. ZIMDAHL. 1980. Allelopathic activity of Canada thistle (*Cirsium arvense*) in Colorado. *Weed Science* 28: 83-86.

STATISTIX INSTITUTE INC. 1985-2009. Statistix Analytic Software. New Version Statistix 9.0: New York.

SUMAN, A., SHAHI, H.N., SING, P., and A. GAUR. 2002. Allelopathic influence of black gram *(Vigna mungo*) seeds on germination and radical growth of some crop plants. *Plant Growth Regulator* 38: 69-74.

TANVEER, A., REHMAN, A., JAVAID, M.M., ABBAS, R.N., SIBTAIN, M., AZRAF UL HAQ, A., IBIN-I-ZAMIR, M.S., CHAUDHARY, K.M., and A. AZIZ. 2010. Allelopathic potential of *Euphorbia helioscopia* L. against wheat (*Triticum aestivum* L.), chickpea (*Cicer arietinum* L.) and lentil (*Lens culiaris* Medic). *Journal of Agricultural and biology* 34: 75-81.

VAN WYK B.E., VAN OUDTSHOORN B., and N. GERICKE. 1997. Medicinal plants of South Africa, 1st Ed, Brizza Publications, Pretoria, p.138.

WINK, M., and B.E. VAN WYK. 2008. Mind-altering and poisonous plant of the world, 1st ed. Briza Publications., Pretoria, p. 409.

WU, H., PRATLEY, J., LEMERLE, D., and T. HAIG. 2000. Laboratory screening for allelopathic potential of wheat (*Triticum aestivum*) accessions against annual ryegrass. *Journal of Agricultural Research* 51: 259-266.

WU, H., PRATLEY, J., LEMERLE, D., HAIG, T. and B. VERBEEK. 2001. Allelochemicals in wheat (*Triticum aestivum*): Variation of phenolic acids in root tissue. *Journal of Chemical Ecology* 27: 125-135.

XUAN, T.D., EIJI, T., HIROYUKI, T., MITSUHIRO, M., KHANH, T.D., and I.M. CHUNG. 2004. Evaluation on phytotoxicity of neem (*Azadirachta indica*. A. Juss) to crops and weeds. *Crop Protection* 23: 335-345.

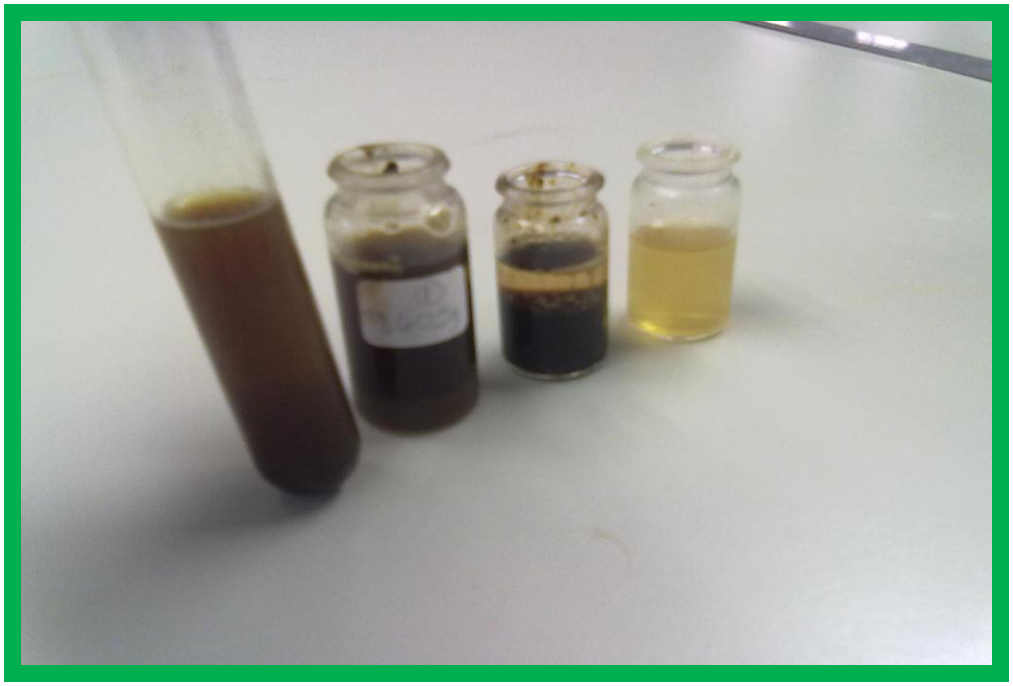
YU, J.Q., and Y. MATSUI. 1994. Phytotoxic substances in the root exudates of *Cucumis sativus* L. *Journal Chemical Ecology* 20: 21-31.

ZUK, A.J., and J.D. FRY. 2006. Inhibition of *'Zenith' zoysiagrass* seedling emergence and growth by perennial ryegrass leaves and roots. *Horticultural Science* 41: 818-821.

APPENDICES

Appendix 3.1 Spotting of methanol, ethanol and acetone extracts of *C. cactiformis* plant on Thin Layer Chromatography (TLC).

Appendix 3.2 Chemical tests for determining the presence of allelochemicals in three extract types.



Appendix 3.3 Experimental layout for seed germination bioassay study.

Appendix 3.4 Experimental layout for seedling growth bioassay study.

Appendix 3.5 Analysis of variance (ANOVA) for germination percentage to ethanol extract of *C. cactiformis* plant.

SOURCE	Df	SS	Percent	F	P ≤
Replication	3	25.00	0.19	1.00	0.43
Treatment	3	12675.0	99.2	507.0	0.00
Error	3	75.00	0.58		
Total	15	12775.0			

Appendix 3.6 Analysis of variance (ANOVA) for hypocotyl length to ethanol extract of *C. cactiformis* plant.

SOURCE	Df	SS	Percent	F	P ≤
Replication	3	0.009	0.054	1.00	0.43
Treatment	3	17.31	99.7	1819.15	0.00
Error	9	0.028	0.16		
Total	15	17.35			

Appendix 3.7 Analysis of variance (ANOVA) for radicle length to ethanol extract of *C. cactiformis* plant.

SOURCE	Df	SS	Percent	F	P ≤
Replication	3	0.07	1.039	1.00	0.43
Treatment	3	6.77	95.8	92.25	0.000
Error	9	0.02	0.33		
Total	15	7.06			

Appendix 3.8 Analysis of variance (ANOVA) for germination percentage to acetone extract of *C. cactiformis* plant.

SOURCE	Df	SS	Percent	F	P ≤
Replication	3	868.75	4.14	0.65	0.60
Treatment	3	16068.7	76.7	12.03	0.001
Error	9	4006.25	19.13		
Total	15	20943.7			

Appendix 3.9 Analysis of variance (ANOVA) for hypocotyl length to acetone extract of *C. cactiformis* plant.

SOURCE	Df	SS	Percent	F	P ≤
Replication	3	1.08	0.36	0.52	0.68
Treatment	3	22.31	7.43	10.65	0.00
Error	9	6.28	0.69		
Total	15	29.69			

Appendix 3.10 Analysis of variance (ANOVA) for radicle length to acetone extract of *C. cactiformis* plant.

SOURCE	Df	SS	Percent	F	P ≤
Replication	3	0.25	5.80	0.65	0.60
Treatment	3	2.90	67.28	7.49	0.00
Error	9	1.16	26.91		
Total	15	4.31			

Appendix 3.11 Analysis of variance (ANOVA) for germination percentage to methanol extract of *C. cactiformis* plant.

SOURCE	Df	SS	Percent	F	P ≤
Replication	3	168.75	1.88	1.00	0.43
Treatment	3	8268.75	92.45	49.00	0.00
Error	9	506.25	5.66		
Total	15	8943.75			

Appendix 3.12 Analysis of variance (ANOVA) for hypocotyl length to methanol extract of *C. cactiformis* plant.

SOURCE	Df	SS	Percent	F	P ≤
Replication	3	0.01	0.10	1.00	0.43
Treatment	3	9.42	99.36	584.00	0.00
Error	9	0.04	0.42		
Total	15	9.48			

Appendix 3.13 Analysis of variance (ANOVA) for radicle length to methanol extract of *C. cactiformis* plant.

SOURCE	Df	SS	Percent	F	P ≤
Replication	3	0.04	0.74	1.00	0.43
Treatment	3	5.16	96.44	111.00	0.00
Error	9	0.13	2.42		
Total	15	5.35			

Appendix 3.14 Analysis of variance (ANOVA) for germination percentage to distilled water extract of *C. cactiformis* plant.

SOURCE	Df	SS	Percent	F	P ≤
Replication	3	300.00	3.15	1.64	0.24
Treatment	3	8650.00	91.05	47.18	0.00
Error	9	550.00	5.78		
Total	15	9500.00			

Appendix 3.15 Analysis of variance (ANOVA) for hypocotyl length to distilled water extract of *C. cactiformis* plant.

SOURCE	Df	SS	Percent	F	P ≤
Replication	3	1.05	5.46	2.19	0.15
Treatment	3	16.74	87.05	34.85	0.00
Error	9	1.44	7.48		
Total	15	19.23			

Appendix 3.16 Analysis of variance (ANOVA) for radicle length to distilled water extracts *C. cactiformis* plant.

SOURCE	Df	SS	Percent	F	P ≤
Replication	3	0.03	1.53	0.12	0.94
Treatment	3	1.10	56.12	4.01	0.04
Error	9	0.82	41.83		
Total	15	1.96			

Appendix 3.17 Analysis of variance (ANOVA) for seedling emergence to extract of *C. cactiformis* plant.

SOURCE	Df	SS	Percent	F	P ≤
Replication	3	0.16	9.05	0.62	0.60
Treatment	5	0.33	18.16	0.75	0.59
Error	15	1.33	72.67		
Total	23	1.83			

Appendix 3.18 Analysis of variance (ANOVA) for seedling height to extract of *C. cactiformis* plant.

SOURCE	Df	SS	Percent	F	P ≤
Replication	3	69.83	12.43	1.04	0.40
Treatment	5	154.80	27.56	1.38	0.28
Error	15	336.88	59.99		
Total	23	561.52			

Appendix 3.19 Analysis of variance (ANOVA) for chlorophyll content to extract of *C. cactiformis* plant.

SOURCE	Df	SS	Percent	F	P ≤
Replication	3	8.22	9.63	0.78	0.52
Treatment	5	24.35	28.51	1.38	0.28
Error	15	52.80	61.84		
Total	23	85.38			

Appendix 3.20 Analysis of variance (ANOVA) for fresh biomass weight to extract of *C. cactiformis* plant.

SOURCE	Df	SS	Percent	F	P ≤
Replication	3	1.00	11.87	1.02	0.40
Treatment	5	2.55	30.17	1.56	0.23
Error	15	4.89	57.94		
Total	23	8.45			

Appendix 3.21 Analysis of variance (ANOVA) for dry biomass weight to extract of *C. cactiformis* plant.

SOURCE	Df	SS	Percent	F	P ≤
Replication	3	0.001	5.26	0.78	0.52
Treatment	5	0.005	26.31	1.38	0.28
Error	15	0.012	63.15		
Total	23	0.019			

Appendix 3.22 Abstract submitted as the oral presentation at the 11th African Crop Science Society Conference, Entebbe-Uganda 14-17 October 2013.

Sebola, M.F., Mafeo, T.P., Mamphiswana, N.D., Mashela, P.W., Bagla, V.P., Mokgotho, P.M. and Khosa, M.C. 2013. Germination bioassay and phytochemical analysis of Cissus cactiformis extract on lettuce seeds. African Crop Science Society Conference Proceedings, Entebbe-Uganda 11: 47-48.

GERMINATION BIOASSAY AND PHYTOCHEMICAL ANALYSIS OF *CISSUS CACTIFORMIS* EXTRACT ON LETTUCE SEEDS

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This study was conducted to investigate allelochemical content of *Cissus cactiformis* and to determine its allelopathic effect based on presence of the phytochemical component on germination of lettuce seeds. Phytochemical analysis of *C. cactiformis* plant extract showed some differences in the allelochemical components of different extracts tested. Saponins, terpenoids, flavonoids, tannins, alkaloids, coumarins, phytosterols were detected in plant materials extracted using acetone, methanol and ethanol. With these extracts the presence of phenolic compounds and cardiac glycoside were not detected. However, in distilled water extract terpenoids, flavonoids, tannins, alkaloids, coumarins and phenolic compounds were detected, with saponins and cardiac glycoside being absent. Lettuce seeds in germination bioassay were exposed to four treatments, *viz*. 0, 0.5, 1.0 and 1.25 mg/ml of the

different extract were laid-out in a completely randomised design replicated four times. In all variables measured, treatments were significantly ($P \leq 0.01$) different for acetone, ethanol, distilled water and methanol extract of *C. cactiformis*. Mean germination %, hypocotyl and radicle length for control were highest when compared to other levels of each extract type. Our results revealed that irrespective of the solvents used, extract of *C. cactiformis* had allelopathic effects on the germination of lettuce seeds.

Key words: Allelopathy, *Cissus cactiformis,* extracts, mean germination percentage, *Lactuca sativa*

Appendix 3.23 Abstract submitted and accepted as the oral presentation at the 1st Africa-International Allelopathy Congress, Sousse-Tunisia 6-9 February 2014.

Sebola, M.F., Mafeo, T.P. and Mamphiswana, N.D. 2014. Seedling growth bioassay and phytochemical screening of Cissus Cactiformis plant extract on lettuce.African-International Allelopathic Congress, Sousse-Tunisia 1.

Seedling growth bioassay and phytochemical screening of *Cissus Cactiformis* extract on lettuce seeds

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Abstract

Some plant crude extracts with bio-pesticidal properties have also been documented for their phytotoxic properties. The present study was carried out to investigate allelopathic effect of *C. cactiformis* plant as bio-nematicide on seedling growth of lettuce as well as profiling its allelochemical compounds. Seedling growth bioassay comprised six treatments, *viz.* 0, 0.5, 1.0, 1.25, 2.0, and 2.25 g crude extract of *C. cactiformis* plant per pot, were arranged in a randomized complete block design, with six replicates inside the greenhouse. All measured variables were not significantly different at $P \leq 0.05$ level. Phytochemical screening of different extracts was determined using thin layer chromatography (TLC) and Chemical test. Results of phytochemical screening of *C. cactiformis* plant extract showed some differences in the allelochemical components of the different extracts investigated. Saponins, terpenoids, flavonoids, tannins, alkaloids, coumarins and phytosterols were found in plant materials extracted using acetone, methanol and ethanol. With these extracts the presence of phenolic compounds and cardiac glycoside were not found. However, in distilled water extract terpenoids, flavonoids, tannins, alkaloids,

coumarins and phenolic compounds were detected, with saponins and cardiac glycoside being absent. The results of this study explained for the first time why the material when used as the post-emergent bio-nematicide within these dosages improved in growth of the tested plant and revealed the allelochemicals responsible for suppressing nematodes.

Key words: Allelochemical compounds, bio-nematicide, bio-pesticidal properties, crude extract, post-emergent