THE HERBICIDAL POTENTIAL OF *Tagetes minuta* AND *Tagetes patula* EXTRACTS AND THEIR RESIDUES ON THE GERMINATION AND GROWTH OF SOME CROP PLANTS.

BY

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Declaration

This is my original work that has not been presented for a degree in any other University

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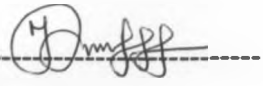

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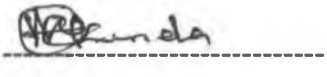

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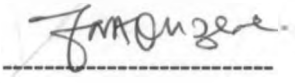

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Dedication

This work is dedicated to Henry Mogote and Ebisiba Kerubo who motivated and encouraged me to accomplish my research work even when I was financially depressed.

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LIST OF ABBREVIATIONS

MGP	mean germination percentage
MGT	mean germination time
T.mRw	*Tagetes minuta* root water extract
T.mLw	*Tagetes minuta* leaf water extract
T. mRm	*Tagetes minuta* root methanol extract
T. mLm	*Tagetes minuta* leaf methanol extract
T. mRt	*Tagetes minuta* root thiophene extract
T. mSr	*Tagetes minuta* seedling residue extract
T. pRw	*Tagetes patula* root water extract
T. pLw	*Tagetes patula* leaf water extract
T. pRm	*Tagetes patula* root methanol extract
T. pLm	*Tagetes patula* leaf methanol extract
T. pRt	*Tagetes patula* root thiophene extract
T. pSr	*Tagetes patula* seedling residue extract
R. I	*Rorripa indica*
E. c	*Eleusine coracana*
P. v	*Phaseolus vulgaris*
C. f	*Capsicum frutescens*

ABSTRACT

The study was conducted to determine the herbicidal effect of *Tagetes patula* and *Tagetes minuta* on the germination and seedling growth of *Eleusine coracana, Rorripa indica, Capsicum frutescens* and *Phaseolus vulgaris*. Seeds of *T. patula* and *T. minuta* were planted on two plots and the seedlings harvested after attaining the height of 7-10 cms. Extracts of water, methanol and thiophenes were obtained from the roots and leaves of the dried samples of *T. minuta* and *T. patula*. Test plant seeds of *Eleusine coracana, Rorripa indica, Capsicum frutescens* and *Phaseolus vulgaris* were subjected to three levels of extract concentration; 20%, 40% and 60% in a completely randomized block design with each replicated three times. The biological activities of these extracts were assessed by examining the reduction in mean germination percentage and the subsequent delay of germination time. Root length, shoot and leaf area were measured to determine the suppression effect of Tagetes extracts on the growth of seedlings of the test plants. The SPSS package was used to analyze the data collected in which analysis of variance and post hoc test using LSD were carried out to explore the impact of each extract on the germination percentage, delay and on seedling growth. The results showed a significant decrease at $p<0.05$ in the mean germination percentage, germination time, root and shoot length. The magnitude of germination inhibition was shown to be proportional to the extract concentration. Germination inhibition and seedling growth suppression was great in the residue, water and methanol treatment compared to thiophene. Roots of the test plants were shown to be the most affected by the extracts, followed by the shoot length, though the leaf area was non significantly affected ($p>0.05$). The sensitivity to these extracts varied among the test plants with *R. indica* showing the lowest mean germination

percentage in all treatment, however, the mean germination time depended highly on the concentration of the extract. The test plants *C. frutescens, E. coracana* and *P. vulgaris* similarly registered a decline in mean germination percentage and an average delay on the germination of the seeds. Therefore, the residues and the crude extracts of water and methanol did show significant reduction effect on the germination and the growth of the seedlings.

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background

Plants are static organisms which cannot escape the pressures caused by abiotic and biotic factors (Burylo *et al.,* 2007). Their interaction with environmental factors may be positive, negative or neutral and this may affect their biochemical, physiological and molecular aspects of growth (Rice, 1984). The negative interactions involve competition which shapes the plant population structure, while positive interaction facilitates the growth of plant populations. Some plants interact with their environment by releasing chemical substances called allelochemicals (Rice, 1984). This phenomenon is called allelopathy and has been defined as the direct or indirect harmful or beneficial of one plant to another throught the release of chemical substances into the surrounding (Ashraf *et al.,* 2008).

Plants biosynthesis a wide range of allelochemicals including; phenolics, terpenoids, alkaloids and flavonoids (Rizvi and Rizvi, 1992) which accumulate in different parts including the leaves, roots, fruits, flowers and bark (Rice, 1984). These allelochemicals are liberated into the environment in a number of ways which involve; decomposition, volatilization, exudation and as leachates (Rice, 1984; Nair *et al.,* 1990). Ones in the soil, allochemicals affect the development and growth of neighboring plants in different ways including germination inhibition and growth (Rizvi and Rizvi, 1992; Tiffany *et al.,* 2004). However, the pattern of germination inhibition and the suppression on earlier seedling growth need to be adequately addressed.

Allelochemicals have been reported to adversily affect different physiological processes which lead to the germination of the seeds (Rice, 1984). Uptake of mineral nutrients from

the soil and the attack of the naturally occurring symbiotic relationship has been the target of these allelochemicals (Rice, 1984; Putnam, 1988). Germinating seeds have been identified to be the most sensitive stage of the plant cycle to the allelochemicals (Leather and Einhellig, 1986). It has been reported that the allelochemicals affect negatively the processes of cell division (Rizvi and Rizvi, 1992; Iganci *et al.,* 2006), protein synthesis and the permeability of the membrane to water absorption (Rice, 1984). Subsequently, the seedlings have been shown to be very sentive to the effect of the allelochemicals (Tobe and Omasa, 2000). The development rates of the roots and the shoot have been shown to be reduced by the allelochemicals (Sajjad *et al.*, 2007). Their studies showed that the roots of the test plants were more sensitive to the allelochemicals than the aerial parts of the seedling. However, more research has to be done to verify if different plant species respond in a similar manner to the action of allelochemicals from the producing plant.

Allelochemicals have been reported to enable allelopathic plants to successfully displace the indigenous plants from their habitat and establish a stable population (Jose and Callaway, 2003). For example, anumber of invasive plants including *Tagetes minuta* has been reported to produce allelochemicals that suppress the germination and growth of *Accasia asak* plants in the field (Arif, 2008). Many plants to have been reported to displace or reduce the germination and growth of other plants include, *Sorghum bicor*, *Lantana camara*, *Helianth annuus* (Rizvi and Rizvi, 1992, Ashraf *et al.,* 2008)

Since these plants have been shown to have influence on the germination and growth of other plants through the action of the allelochemicals, then they can be utilized to manage the weeds that have become resistant to the herbicides (Yang *et al.,* 2007). For example, some weeds including *Phalaris minor* and *Echinochloa coloca* have developed

resistance against selective herbicides like isoproturon and -propanil, and their control has dropped from 78% to 27% within a time of 3 years (Malik and Singh, 1995).

Recent research findings have shown that allelopathy holds great prospects for the management of weeds (An *et al.,* 1998; Marcias, 1995). Study by Singh *et al.,* 2003 demonstrated a number of crop plants with allelopathic potential that can be used as cover crops and as mulch to manage the weeds. Alsaadawi, (2007) repoted that, extracts from *Sorghum bicolor* exhibited selectivity to particular weeds for example; Purple nutsedge and the research was in agreement with the work done by Mahmood and Cheema, 2003. Therefore, structural modifications or synthesis of analogues based on the allelochemicals can be carried out to increase their application as natural herbicides (An *et al.,* 1998b; Marcias, 1995).

Allelopathy can be employed as an alternative weed control strategy which is environmentally safe, conserves the available natural resources including water and mineral salts and also mitigates the problems raised by synthetic chemicals (Rizvi and Rizvi, 1992). The application of allelopathy in weed management varies; some plant can be cropped or intercropped with other crops (Iqbal and Cheema, 2007b), while some may be spread on the surface as mulch or incorporated into the soil to suppress the weeds (Cheema *et al.,* 2000; Sati *et al.,* 2004). Similarly, aqueous extract can be made from the allelopathic plants and applied as herbicides (Javaid and Anjuma, 2006).

The present study involves two allelopathic plants which belong to the Asteraceae family; *Tagetes patula* L. and *Tagetes minuta* L which have been observed to accompany most of the crops in the field. *Tagetes* plants are considered as invasive species originating from Southern America (Soule, 1993) and they are characterized by a pungent smell, indicating the presence of allelochemicals. Their spread and proliferation capacity is attributed to a number of factors including; the production of numerous seeds and

presence of allelochemicals, for example; phenolics, flavonoids, acyclic, monocyclic and bicyclic terpenes, sesterpernes and polyacetylenes (Rodriquez and Mabry, 1977). *Tagetes minuta* often grow in disturbed areas during early successional stages and it is an erect annual herb reaching 1 to 2 m. Leaves are slightly glossy green, and are pinnately dissected into 4 to 6 pairs of pinnae. Leaf margins are finely serrate and there are typically 3 to 5 yellow-orange ray florets, and 10 to 15 yellow-orange disk florets per capitula. The heads are small, 10 to 15 mm long, and including ray florets, 10 to 20 mm in diameter. The heads are borne in a clustered panicle of 20 to 80 capitula. The dark brown achenes are 10 to 12 mm long; with a pappus of 1 to 4 tiny scales and 0 to 2 retrosely serrulate awns which are 1 to 3 mm long.The French marigolds (*Tagetes patula*) are small bushy plants that are about 6-12 in (15-30 cm) in height. The flowers are up to 2 in (5 cm) across and are composed of a dense arrangement of "rays" that come in yellow, orange and a unique bronze colour.

The allelopathic activities of these two plants were tested on four plants *Eleusine coracana, Phaseolus vulgaris, Capsicum fruitescen* and *Rorripa indica.* These plants were used used in this study since their germination rate and growth rate are known and therefore the allelochemical effect of the extracts can be noticed easily.

1.2 Problem statement

Most weeds have become resistant to the synthetic herbicides, thus challenging the farmers and researchers to seek for an alternative weed control measure. The continued survival of the weeds within the population of economic plants, greatly reduce economic crop product and at the same time lowers its quality. Consequently, the persistent use of herbicides poses some environmental risks including, non-selectivity and residual effect. Therefore, natural herbicides are recommended for the management of weeds and this involves the use of allelopathic potential of aromaric plants. Although, most of these plants are weeds, they have the ability of suppressing the germination and growth of other weeds in the fields. The challenging issue involves growing of allelopathic plants, stage of utilizing its allelopathic property and the most effective part to be used for the weeds management. Though, a lot of research has been done on allelopathy in plants, little work has been done to discriminate the influence of allelochemicals on economic crops. The present study focuses on the influence of *Tagetes minuta* and *Tagetes patula* on the germination and growth of selected economic crops and weed in laboratory conditions. The application of various extracts on the test plant seeds and results on germination and growth reflects allelopathy of *T. minuta* and *T. patula* species. The future application of *T. minuta* and *T. patula* extracts and residues in controlling weeds, largely depends on the influence on the economic crops.

1.3 Overall objective

To investigate the herbicidal potential of the various extracts from *Tagetes minuta* and *Tagetes patula* on the germination and growth of *E coracana, P. vulgaris, C. frutescens* and *R. indica* seeds.

1.3.1 Specific objectives

1. To investigate the effects of various extracts from *T. minuta* and *T. patula* on the germination of *E coracana, P. vulgaris, C. frutescens* and *R indica.*
2. To demonstrate the sensitivity of target plant growth parameters; root, shoot or leaf area to various extracts from *T. minuta* and *T. patula.*
3. To compare the plant herbicidal effect between the roots and leaves of of *T. minuta* and *T. patula* with the greater.
4. To investigate the residual effect of *T. minuta* and *T. patula* on the germination and growth of the test plants.

1.4 Hypotheses

1. Extracts and residues of *T. patula* and *T. minuta* significantly reduce the germination and growth of the test plants.
2. Test plant seeds and seedlings respond differently to the various extracts from *T. minuta* and *T. patula.*

1.6 Justification

A considerable body of information has accumulated implicating allelopathy as the most important form of association that contributes to declining performance of crop plants. However, allelochemicals from these plants have been shown to have the ability to effectively control weeds in fields.This calls for a thorough research method to give a convincing proof that some of the common weeds can be utilized to suppress other weeds. Therefore, *Tagetes* which grows together with most of the crop plants in the field, produce a diversity of allelochemicals that reduce the germination and growth of other plants.

Allelochemicals can be extracted from these plants using various extracts and their biological activities tested in laboratory conditions to verify their effectiveness in suppressing other plants in ideal environments. Both, water, methanol, mixture of solvents and residues of decomposing plants residues can be employed as a means of extracting bioactive compounds from *Tagetes minuta* and *Tagetes patula*. The effect of these extract on plants can be assessed by conducting bioassay experiments so to observe if they affect both crop plants and other weeds or they can selectively affect the weeds.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Diversity of allelochemicals from plants and their influence on other plants.

Plants live in complex environments where many biotic and abiotic factors limit or promote productivity (Burylo *et al.,* 2007). Temperature, extreme rains, nutrients, diseases, insect and competition from other plants can influence each other, giving rise to a complex environmental conditions to which plants must adapt. The interaction of these factors with plants influences the biochemical, physiological, molecular and the productivity of plants (Rice, 1984; Ashraf, 2008). Therefore, plants have evolved adaptive mechanisms; both physiological and chemical of enduring the stress.

The term allelopathy was defined by (Torres *et al*., 1996; Ashraf *et al.,* 2008) as any process which involves the production and release of allelochemicals from plants and other micro-organisms that influence the growth and development of agricultural plants. These allelochemicals are distributed in different plant families, including; barley (*Hordeum vulgare*), clovers (*Trifolium* spp., *Melilotus* spp.) oats (*Avena sativa*) pearl millet (*Pennisetum glaucum*), rice (*Oryza sativa*) rye (*Secale cereale*), sorghums (*Sorghum spp.*), sunflower (*Helianthus annuus*), sweet potato (*Ipomoea batatas*) and wheat (*Triticum aestivum*) have exhibited allelopathic effects on the germination and growth of other plants (Weston, 1996).

The allelochemical compounds are produced by various biosynthetic pathways and are stored in several parts of plants including the roots, rhizomes, leaves, stems, pollen, seeds and flowers (Chon and Kim, 2002; Rodriquiz and mabry, 1977). These chemicals are released into the environment by root exudation, leaching from above ground parts, volatilization and decomposition of plant material (Rice, 1984).

The entry and accumulation of allelochemicals may persist for long in the soil leading to changes in microbial activities; chemical characteristics of the germination medium. Bogatek *et al.,* (2006) revealed increasing allelochemical concentration lead to higher germination inhibition effect. For example, when susceptible plant seeds are subjected to various allelochemical extracts, the germination rate may be reduced depending on the concentration of the extract (Randhawa *et al.,* 2002). Similarly, Sajjad *et al.,* 2007 showed the effect of allelochemicals from *Cassia angustifolia* on the number of leaves in cereal plants, however the leaf area which is a measurable parameter that determines the amount of light captured, synthesis of assimilates and eventual plant biomass production is rarely taken into consideration.

Allelopathy involves a wide spectrum of chemicals ranging from simple hydrocarbons, aliphatic acids to complex poly-cyclic structures (Rice, 1984; Putnam and Tang, 1986). These allelochemical compounds can be categorized further, into simple water-soluble organic acids, polyacetylenes, phenols, benzoic acid, flavonoids, tannins, terpenoids, sulphides and glucosides (Akinpelu *et al.,* 2009; Whittekar and Feeney, 1971; Rice, 1984 and Putnam and Tang, 1986). The production of allelochemicals by allelopathic plants has been suggested to increase in response to environmental stress (Einhellig, 1996; Inderjit and Del Moral, 1998a).

Reigosa *et al.,* (1999) revealed that both biotic and abiotic factors including the light, nutrient availability, water availability, and pesticides may influence the impact of allelochemicals on the receiver plant. Similarly, environmental stress has been shown to increase the sensitivity of target plants to allelochemicals (Einhellig, 1996; Reigosa *et al.*, 1999). Therefore, the effect of allelochemical in soil to the receiver plant may not be directly related to the actual concentration of the allelochemical in soil.

Transformation of the allelochemicals also takes place due to biogeochemical processes resulting into the formation of more or less phytotoxic compounds (Blum *et al.*, 1993). Some allelochemical compounds are easily transformed, while others may persist for a long period in soil, because of their anti-microbial activity (Nair *et al.,* 1990). Reports by Inderjit and Dakshini, 1994, revealed that the concentration of allelochemicals in the soil medium depends on the density and age of the allelopathic plant.

In demonstrating allelopathy, the most susceptible stage of the target plant is always taken into account (Leather and Einhellig, 1986). They suggested that seed germination and seedling development are measurable parameters and that are very susceptible to allelochemicals. Chaves and Escudo, (1997); El- Khatib, (1998) suggested that in studying allelopathy some factors including germination delay cotyledon and root size reduction should also be considered. Similarly, various studies have also shown that allelopthic plants can be used in the management of weeds (Narwal *et al.*, 1998) for example, some of the related management strategies include; residues and rotational sequence in the suppression of weeds in the cropping system (An *et al.,* 1998). Macias, (1995) demonstrated that, allelochemicals can be extracted from some plants and modified to bio-herbicide. This implies that allelopathic properties of crop species can be considered as a supplement to other weed management strategies (An *et al.,* 1998). The weed management strategies involve the exploitation of the allelochemicals which can selectively suppress the weeds in a cropping system.

2.2 The influence of allelochemicals on growth of other plants.

Allelopathy is wide spread among plant species and it often affects the growth of neighboring plants negatively (Brown and Morra, 1995; Mathiassen *et al.,* 2006). For example, Rye (*Secale cereale*) has been shown to influence the growth of succeeding plant species in the field through the release allelochemicals (Barnes *et al.*, 1985). It has been reported that, decomposing rye plants release various allelochemicals including phenolics (beta-phenyl-lactic acid (PLA) and hydroxamic acids DIBOA (2,4-dihydroxy-1,4(2H)- benzoxazin-3-one) and BOA (2(3H)-benzoxazolinone into the soil (Narwal, 1994). These allelochemicals were reported by Perez and Ormemeno, 1991 to cause a reduction in the germination, stunted growth, retardation of root development and chlorosis of the leaves of oat, *Avena fatua.* Therefore, the residues of the rye plant have been employed as mulches or cover crops in no-tillage cropping systems to suppress certain weed species (Barnes and Putnam, 1986). Creamer *et al.,* (1996) indicated that the physical suppression of rye was responsible for the reduced emergence of two weedy species, eastern black night shade *(Solanum ptycanthum)* and yellow foxtail (*Setariaglauca).*

Similarly, wheat residues have been shown to suppress the germination of weeds due to the production of allelochemicals which including phenolic acids and simple acids that have been identified in their residues (Narwal, 1994). The allelopathic activity of the wheat extracts against *Lolium rigidum* has been found to correlate to the total phenolic content in the tissue of the wheat cultivars (Wu *et al.,* 1998). Hydroxamic acids have also been identified in shoot and root tissue of wheat. The most abundant of these acids in wheat tissues is DIMBOA and are effective in controlling weeds (Copaja *et al.,* 1999).

Most of the wheat allelochemicals have been shown to inhibit root growth and seed germination in wild oat, *Avena fatua* (Perez, 1990).

More of the allelochemical compounds including; phenolic compounds, for example ferulic, vanillic and phydroxybenzoic acids that have been identified in the water and methanol extracts of barley plants (Borner, 1960). Lovett and Hoult, 1995 isolated two alkaloids; gramine (N,N-dimethyl-3-amino-methylindole) and hordenine (N,N-dimethyltyramine) which play an important role in allelopathic activities.

The screening of rice has revealed several allelopathic compounds including, phenolic acids 3-hydroxybenzoic acid, 4-hydroxy-benzoic acid, 4-hydroxyhydrocinnamic acid, 3,4-dihydroxyhydrocinnamic acid and 4-hydroxyphenylacetic acid in water extracts (Olofsdotter *et al.,* 1997).These allelochemicals have been shown to act selective against *Purple ammannia* (Olofsdotter *et al.,* (1997). Therefore, allelochemicals which act selectively on plants can be isolated and applied as natural herbicides in the control of weeds.

Tagetes patula and *Tagetes minuta* possess a diversity of allelopathic compounds, for example monocyclic and bicyclic monoterpenes, sesquiterpenes, flavonoids, thiophenes (Rodriguez and Mabry, 1977) which accumulate in different parts of these plants. These compounds cause various biological effects on several organisms including; trematodes (Grainge and Ahmed, 1988) and the germination and growth of other plants (Kil *et al.,* 2002). The quantative effect of these extracts from the different parts of these plants need to be compared so as to identify the part with substantial of the allelochemicals that can be used to control other weeds.

2.3 Allelochemicals with herbicide effect on plants.

Various natural products including; phenolics, alkaloids, terpenoids, essential oils and flavonoids are involved in allelopthic activities (Einhellig and Leather, 1988). These compounds have been isolated and identified from different plants families (Putnam and Tang, 1986). Similarly, various researchers have conducted bioassay experiments using the natural plant extracts to determine their allelopathic potential (Rice, 1984). Phenolic compounds including salicylic acid and p-hydroxybenzoic acid have been reported to be very effective in controlling weeds (Duke *et al.,* 1997).

Some alkaloids including; colchicines, vinblastine, and terpenoids have been shown to reduce the process of mitosis and eventually stop plant growth (Rice, 1974). Rizvi and Rizvi, 1992 showed that alkaloids caused a reduction in amylase activity of germinating seeds of *Amaranthus spinosa* thereby limiting energy supply to the actively dividing embryonic cells.

It has been also reported that allelochemicals can alter the rate at which ions are absorbed by plants (Rice, 1974). For example phenolics have been shown to inhibit the absorption of ions and at the same time cause malformation of chlorophyll (Marsie and Singh, 1988). Similarly, allelochemicals have been shown to alter the membrane permeability of the root hair cells, reducing the rate of water absorption, conductivity and translocation of materials and thereby reducing shoot length (Ashraf *et al.,* 2008; Rice, 1984; Harper and Balke, 1981). In other cases, some allelochemicals have been shown to targets photosystems and modify the electron carriers thus inhibiting the process of photosynthesis, while other chemicals inhibit respiration thereby reducing energy production (Rice, 1974). Barnes and Putnam (1986) revealed that allelochemicals affect different plant growth systems, and are present in both weeds and crop plants.

On the other hand, polyacetylene compounds for example thiophenes are abundant in the roots of Asteraceae plant family (Ilaria *et al.,* 2009; Ketel, 1987). These compounds have been shown to affect the germination of various seeds, for example Alpha-terthienyl (**α-T**), phenylheptatriyne (PHT), were tested for their herbicidal potential against four seedling species (*Asclepias syriaca* L., *Chenopodium album* L., *Phleum pratense* L. and *Trifolium pratense* L.). Germination of seeds was found to be sensitive to α-T and PHT. Therefore, thiophenes from *Tagetes* roots warrant future field trials to assess its potential as a natural weed-control agent (Ilaria *et al.,* 2009, Ketel, 1987; Campbell, *et al.,* 1982). However, the fate and actual modes of action of these compounds are not well understood; whether these compounds inhibit the emergence of seeds weeds and therefore recommended more research to be done on these compounds. Therefore, in the present study, the effect of extracts from *Tagetes* species on germination of the test plants was assessed throught the germination percentage, germination time, leaf area, shoot and root lengths.

2.4 Allelopathy as an alternative strategy for managing weeds.

The utility of allelopathy in the management of weeds was pioneered by Putnam and Duke, 1974. Their work suggested that intercrops and rotation of cover crops can be intergreted for effective weed management. The work done by Moyer *et al.,* 2000; Petersen *et al.,* 2001; Sene *et al.,* 2001 revealed that cover crops and their residues reduced the emergence of weeds in fields. Burgos and Talbert, (2000); Nagabushana *et al.*, (2001) reported that the cover crop residue on the soil surface contributes to weed suppression coupled to the phytotoxins released from decomposing residues. Similarly, Putnam 1988; Weston, 1996 Bertin *et al.,* 2003; Weston, 1990 have demonstrated that

fine leaf fescues and mixtures of forbs and grasses have the ability to suppress the germination of small seeded weeds.

Various studies have been conducted to evaluate weed suppression effect by various plant species, including Sorghum *Sorghum bicolor* which is known to provide good weed killing capacity (Putnam, *et al.,* 1983). The activity of sorghum root exudates from 100 cultivars was examined by Alsaadawi *et al.,* 1985 and tested on the germination and seedling development of Pigweed (*Amaranthus retroflexus*). They observed considerable toxicity through aqueous extracts and decaying materials. Alsaadawi, (1992) isolated and identified the phenolic compounds occurring in sorghum plants that are very active in causing the germination inhibition effect.

Studies by Chou, 1990; Rimando and Duke, 2003 indicated that rice (*Oryza sativa*) planted twice a year in a monoculturte system reduced the second crop yield by about 25% in areas of poor water drainage. Decomposition of rice residues releases phytotoxins into the soil leading to the suppression effect on the growth of the subsequent crops. They showed that the phytotoxicity of these allelochemicals in the soil increased with an increase in amount of straws (Chou, 1990; Rimando and Duke, 2003).

Study by Singh *et al.,* 2003 demonstrated a number of crop plants with allelopathic potential that can be used as cover crops and as mulch to manage the weeds; barley (*Hordeum vulgare*), sunflower (*Helianthus annua*) (Rice 1984; Singh *et al.*, 2001), fine fescues , Weston, 1990, mustards (Petersen *et al.* 2001; Siemens *et al.,* 2002). In many cases aqueous extract can be made from the allelopathic plants (Iqbal and Cheema, 2007; Javaid and Anjuma, 2006)

Therefore, allelopathy can be employed as an alternative method for managing weeds without polluting the environment (Rizvi and Rizvi, 1992). Various suggestions have

been proposed including; intercrops (Iqbal and Cheema, 2007), mulch (Cheema *et al.,* 2000), residues (Sati *et al.,* 2004) in the suppression of weeds. Allelopathic overcomes the challenge of resistance to synthetic herbicides in the field by weeds (Prather *et al.,* 2000). For example, the first case of herbicide resistance was against triazine herbicide in common groundsel (*Senecio vulgaris*) that was reported 1968 from U.S.A. (Ryan, 1970).

More weeds have been shown to develop the resistance to synthetic herbicides including, *Cornyza Canadensis*, *Sorghum helepense, Amaranthus palmeri* and *Ambrosia trifida* to glyphosate in soybean fields where glyphosate (Valverde and Gressel, 2006; Barnes, *et al.,* 2003). Canary grass *Phalaris minor* and *Jungle rice, Echinochloa colona* have reported to have developed resistance against Isoproturon, propanil Clodinofop and Sulfosulfuron (Mahajan and Bvar, 2001).

Continuous application of the same herbicide or different herbicides with the same mode of action creates selection pressures that allow resistant populations to develop exclusive and site selection mechanisms of resistance (Dekker and Duke, 1995). On the other hand site of action resistance includes altered site of action such that it is no longer susceptible to the herbicide. This has been observed in *Lactuca sativa* which are resistant to sulfonyl urea herbicides (Eberlein *et al.,* 1999). Therefore, more work has to be done on bioactive compounds that have the ability to suppress the germination and growth of herbicides resistant weeds. However, few attempts have been done on some plants for their potential in the management of weeds that are resistant to herbicides. For example, Wu *et al.,* 1998, evaluated the toxicity of aqueous extracts from wheat shoot residues on herbicide resistant ryegrass. They found that the wheat extract significantly inhibited the germination and root growth of the rye grass. Therefore, allelochemicals can be modified

to form new bioherbicides with different modes of action to counteract the resistance of these weeds (Wu *et al.,* 2003)

Since phytochemical studies have revealed that *Tagetes* plant species contain diversity of allelochemicals in the leaves compared to the roots (Hogstad *et al.*, 1984, Ketel, 1987 Rodriquez and Mabry, 1977). Then possibility of utilizing *T. minuta* and *T. patula* extracts and residues as herbicides involves a bio-ecological phase whereby allelopathic interactions must be recognized and demonstrated both in the field and laboratory conditions through bioassay experiments (Putman and Duke, 1978). Studies by Arif, 2008; Kil *et al.*, 2002; Kiran *et al.,*1995 have demonstrated that aqueous extracts obtained from leaves, stem and roots *Tagetes minuta* have the ability to reduce some plants for example *Acacia asak, Lotus comiculatus var. japonicas* and *Lactuca sativa.*

It has been shown that decomposition of plant material, releases allelochemicals into the soil (Nair *et al.,* 1990), which must accumulate and persist in the soil in order to induce the allelopathic suppression on the germination of plant seeds. Bogatek *et al.,* 2006 revealed increasing the amount of decomposing materials, reduced the germination of the seeds of significantly. Study by Daizy *et al.,* 2007 showed that powdered leaves of *Tagetes minuta* significantly reduced emergence and growth of both the weed species in rice plantations.

Owing to the richness of allelochemicals in *T. minuta* and *T. patula* may play a very important role in weed management through allelopathic interactions.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study site.

The study was conducted at the National Agricultural Research Laboratories (NARL) substation of Kenya Agricultural Research Institute (KARI) in Nairobi. *Tagetes minuta* and *T. patula* seeds were planted in the field at the station, while germination experiments were conducted in Weed Science Laboratory. The extraction procedures from the donor plants were carried out in the Plant physiology Laboratory of the School of Biological Sciences, University of Nairobi.

3.2 Source of Experimental materials and seedling establishment

Seeds of *Phaseolus vulgaris, Capsicum frutescens* and *Eleusine coracana* were commercially purchased from the Kenya seed company outlet in Nairobi, while seeds of *Rorripa indica* were collected from the NARL- KARI garden. The seeds of *Tagetes minuta* and *Tagetes patula* were collected along the Kangundo highway, Nairobi.

Two plots, each measuring 30 m by 10 m were identified and primary cultivation was done to remove all plant roots and then, each plot was divided into two subplots of 15 m by 10 m as shown in table 3.1. Seeds of *T. minuta* and *T. patula* were randomly broadcasted on the plots and were covered by thin layer of soil by dragging the farm rake over the ground. The plots were irrigated every two day. Germination occurred on the fifth day of planting, complete germination was noted on the ninth day. Sampling started on the fifth day after planting. The seedlings of *T. minuta* and *T. patula* were uprooted randomly from subplot 1 and 3 on reaching a height of 6-8 cms. Subplot 2 and 4 seedlings were allowed to grow to maturity.

Table 3.1 Field Layout of the *T. patula* and *T. minuta*

Main plot for *T. patula*			Main plot for *T. minuta*	
Subplot 1	Subplot 2		Subplot 3	Subplot 4
Uprooted at seedling stage for screening and bioassay	Allowed to complete the life cycle for the multiplication of the species		Uprooted at seedling stage for screening and bioassay	Allowed to complete the life cycle for the multiplication of the species

From the harvested plants, roots and leaves were excised and then dried under the shade for three weeks. The dried samples were then ground into powder form using the blender and packaged for further analysis.

3.3 Tagetes Extracts

3.3.1 Water extract

Three concentrations of 20% ($200gL^{-1}$,); 40% ($400gL^{-1}$) and 60% ($600gL^{-1}$) were prepared from the samples using water. Powdered plant samples from the roots and leaves of *T.minuta* and *T. patula* were weighted at 200g, 400g and 600g and each was placed in the conical flask containing one litre of distilled water (w/v). The samples were left standing for 48 hours and the extracts were obtained through filtration using the filter papers (Whatmann No. 1). The extraction procedure was repeated three times for each plant organ so as to obtain enough extract for the bioassay experiments.

3.3.2 Methanol extract

Three concentrations of 200 g L^{-1} (20%), 400 g L^{-1} (40%), and 600 g L^{-1} (60%) were prepared by soaking 20g, 40g and 60g of powdered leaf and root from *T. patula* and *T. minuta* in 100 ml of analytical grade methanol for 48 hours at room temperature. The mixture was swirled and filtered through the Whatmann No. 1 filter paper. Excess methanol was removed from the extract through evaporation by using Rota vapor equipment set at 35^0C and the resultant extracts were stored in the refrigerator.

3.3.3 Thiophenes extract

Three extract concentrations of thiophene were prepared from the roots of *T. patula* and *T. minuta* plants according to the procedures given by Croes *et al.,* 1989. A twenty percent extract was prepared in the dark and at room temperature by soaking 20 grams of each root sample in 100 ml of 70% ethanol for 24 hours. This mixture was swirled and then filtered through the Whitman No. 1 filter papers and the solvent was removed by evaporation. The resultant extract was then added to the hexane and butylmethylether in the ratio of 1: 1 and then centrifuged for 10 minutes at 3000 $revmin^{-1}$ and the top phase removed and evaporated under reduced pressure at 40^0 C. The same procedure was followed in preparing 40% and 60% concentrations.

3.3.4 Residue regimes

Three residue regimes (Sn1, Sn2 and Sn3) of *T. minuta* and *T. patula* were established by incorporating seedlings into the soil in the germination plates. In the first residue regime (Sn1), 200 seeds of *T. patula* and *T. minuta* were sown in the germination plates and were allowed togerminate and establish a height of 6-8 cms. The seedlings were uprooted and then incorporated into the soil medium. Residue regime two (Sn2), same

number of seedling of *T. patula* and *T. minuta* were established on same germination plate for two seasons. In Sn3, the seedlings of *T. minuta* and *T. patula* were established and incorporated in the same plate for three seasons before planting the test plants.

3.4 Experimental Design.

A total of four experiments based on the type of extract; water, methanol, thiophene and residue were conducted in the laboratory in a randomized complete block design and the treatments were replicated three times. For each experiment, 192 germination plates measuring, 12 cms by 6 cms were filled to two thirds with sterile soil pre-heated at a temperature of 105°C for 5 minutes in the oven. The plates were then divided into two groups of 96 of according to the source of extract plant; *T. minuta* and *T. patula*. Within the donor plants, the plates were blocked into two groups of 48 based on the leaf and root extract treatments. Twelve plates were randomly assigned to each extract concentration from both leaf and root organs of which 3 were allocated to each test plant and were labeled as shown in table 3.2.

Filter papers presoaked in the appropriate extract concentration (0%, 20%, 40% and 60%) of water, methanol and thiophenes were placed to cover the soil in the germination plates before sowing of the test plant seeds as shown in table 3.2. Control treatments were set in which distilled water was applied. For the residue experiment; Forty eight germination plates with each measuring 20cms by 20 cms and 8 cms in depth were filled with wet subsoil to two thirds. The plates were then divided into two categories of 24 germination plates and assigned randomly for the planting of *T. minuta* and *T. patula*. The two groups were further divided into 3 groups for the three residue regimes (Sn1, Sn2 and Sn3).

Table 3.2 General Experimental design

Donor plant	Part extracted	Extract concentration (12 plates per conc')	Test plant (3 plates per conc' treatment)			
			E. C	*P. v*	*C. f*	*R. i*
T. minuta (96 plates)	Root (48 plates)	60%				
		40%				
		20%				
		0%				
	Leaf (48 plates)	60%				
		40%				
		20%				
		0%				
T. patula (96 plates	Root (48 plates)	60%				
		40%				
		20%				
		0%				
	Leaf (48 plates)	60%				
		40%				
		20%				
		0%				

E. c = *Eleusine coracana*; P. v = *Phaseolus vulgaris*; C. f = *Capsicum fruitescens* and R. i = *Rorripa indica.*

Each box ▭ represents a germination plate for each extract concentration.

3.4.1 Effects of water extracts of *T. minuta* and *T. patula* on seed germination.

Seeds of *P. vulgaris*, *C. frutescens*, *E. coracana* and *R. indica* were presoaked in water extracts of the root and leaf from *T. minuta* and *T. patula* at concentrations of 20%, 40% and 60% for one hour before sowing. Forty five seeds of each test plant were transferred to 3 germination plates containing wet sterile soil covered by moistened filter papers at a rate of 15 seeds per plate. The seeds were laid at equal intervals of 2 cms between the rows and 1 cm within the lines on the filters presoaked in 20ml of extracts at different concentrations. A second filter paper soaked in the same concentration of the extract was laid over the seeds. Then, 20 ml of the extract was applied to the same plates every 3 days. Germination was signified by the protrusion of the radicle and the number of seeds for each test plant species that germinated was recorded daily, until no further germination was observed. Also the germination time in days for each test plant was recorded from each extract treatment.

3.4.2 Effects of methanol extracts of *T. minuta* and *T. patula* on seed germination.

In this experiment, seeds of *P. vulgaris*, *C. frutescens*, *E. coracana* and *R. indica* were soaked in methanol extracts from the leaves and roots of *T. minuta* and *T. patula* at concentrations of 20%, 40% and 60% separately. Forty five presoaked seeds of each test plant species were transferred to three germination plates containing wet sterile soil covered by moistened filter papers moistened in 20 ml of the extract concentrations at a seed rate of 15 per germination plate. The seeds were laid at equal intervals of 2 cms between the rows and 1 cm within the lines and were covered by a second filter paper soaked in the same extract at same concentrations. Subsequently, 20 ml of these extract concentrations were applied to the same plates after every 3 days and the number of seeds

for each species that germinated and the time of germination were recorded daily until no further germination was observed.

3.4.3 Effects of thiophenes extract of *T. minuta* and *T. patula* on seed germination

Seeds of *P. vulgaris*, *C. frutescens*, *E. coracana* and *R. indica* were presoaked in thiophene extracts of the root from *T. minuta* and *T. patula* at concentrations of 20%, 40% and 60% for one hour before sowing. Forty five seeds of each test plant were transferred to 3 germination plates containing wet sterile soil covered by moistened filter papers at a rate of 15 seeds per plate. The seeds were laid at equal intervals; 2 cms between the rows and 1 cm within the lines on the filters presoaked in 20ml of extracts at different concentrations and were covered by a second filter paper soaked in the same extract at same concentrations. Subsequently, 20 ml of these extract concentrations were applied to the same plates after every 3 days until germination.

3.4.4 Influence of residues of *T. minuta* and *T. patula* on seed germination.

In this experiment, three residue regimes designated Sn1, Sn2 and Sn3 were established from *T. minuta* and *T. patula* seedlings as described in 3.3.4. Three furrows of 5 mm in depth and 2cm a part were made across each germination plate and 15 seeds of each test plants were sown on the plates randomly. The germination plates were watered every three days with 20 ml of distilled water and were allowed to establish on the benches.

3.5 Influence of extracts and residues of *T. minuta* and *T. patula* on seedling growth.

3.5.1 Leaf area (LA)

The average leaf area was found by selecting at random nine seedlings of each test plant species from each extract concentrations of 20%, 40% and 60%. Two leaves from each plant were selected at random selected and clipped on the grid paper. The margin of each leaf was traced and the area was calculated by summing the number of whole squares and half squares enclosed within the area. The average leaf area for each species per extract treatment was calculated by summing the areas of the leaves from the nine plants and divided by the number of leaves for each species.

3.5.2 Shoot length (SL)

Nine seedlings of each test species were randomly selected from three germination plates for each extract concentration. The seedlings were carefully uprooted and their heights measured using a 30 cm ruler from the base of the stem to the apical meristem. The lengths were averaged for each test plant of the 9 seedlings.

3.5.3 Root length (RL)

The effects of water, methanol, thiophene extracts and residue from the leaves and roots of *T. minuta* and *T. patula* at concentrations of 20%, 40% and 60% on the root length of *E. coracana, P. vulgaris, C. frutescens and R. indica*, was estimated by comparing with those of the control treatment. For the purpose of measuring the root length, 9 seedlings of each species were randomly selected from three plates under the same concentrations. Then root length of each test species seedling was measured from the base of the stem to the tip of the tap root and then the average of the 9 seedlings of each species per treatment was calculated.

3.6 Data analysis

The data obtained for germination inhibition is presented in mean germination percentage (MGP) and mean germination time (MGT) as used by (Maryam nad Mansour, 2006; Kil and Yun, 1992). Similarly, the average leaf area (LA), root and shoot lengths of the treated seedlings were compared to the control treatment. All data were statistically analyzed using the SPSS package in which analsis of variance was carried out to compare the means with the significant amount in means identified by post hoc test using the LSD at $P = 0.05$.

CHAPTER FOUR

4.0 RESULTS

4.1 Effects of water extracts of *T. minuta* and *T. patula* on the germination of seeds.

The germination response of *E.coracana, P. vulgaris, C. frutescens* and *R. indica* seeds to aqueous extracts from the roots and leaves of *T. minuta* and *T. patula* are shown in figures 4.1a, b, c and d. Highest mean germination percentages (MGPs) for *P. vulgaris, E. coracana, C. frutescens* and *R. indica* were recorded in the control treatment with 97%, 96.0%, 94.6% and 85% respectively. However, the MGP of each test plant declined significantly at $P<0.05$ on the application of the water extracts from the roots and leaves of *T. minuta* and *T. patula* when compared to the control. At concentration of 20% aqueous extract from the roots of *T. minuta*, the MGP values of 93.5%, 91.3%, 87% and 45.5% were obtained for *P. vulgaris, E. coracana, C. frutescens* and *R. indica* respectively, while 95.2%, 87.8%, 85.6% and 42.3% MGPs were obtained in the *T. patula* root water extract treatment. The MGPs of *P. vulgaris, E. coracana, C. frutescens* and *R. indica* seeds in *T. minuta* leaf water extracts were 91.1%, 83%, 78.7% and 41.5%, while in *T. patula* leaf water extract the MGPs of 93.5%, 85.4%, 76.6% and 37.2% were recorded respectively.

At a concentration of 40%, *P. vulgaris, E. coracana, C. frutescens* and *R. indica* registered MGPs of 90.4%, 80.4%, 83.0%, and 33.3% in *T. minuta* root water as

compared to MGPs of 91.1%, 88.4%, 76.3% and 40.7% in *T. patula*.

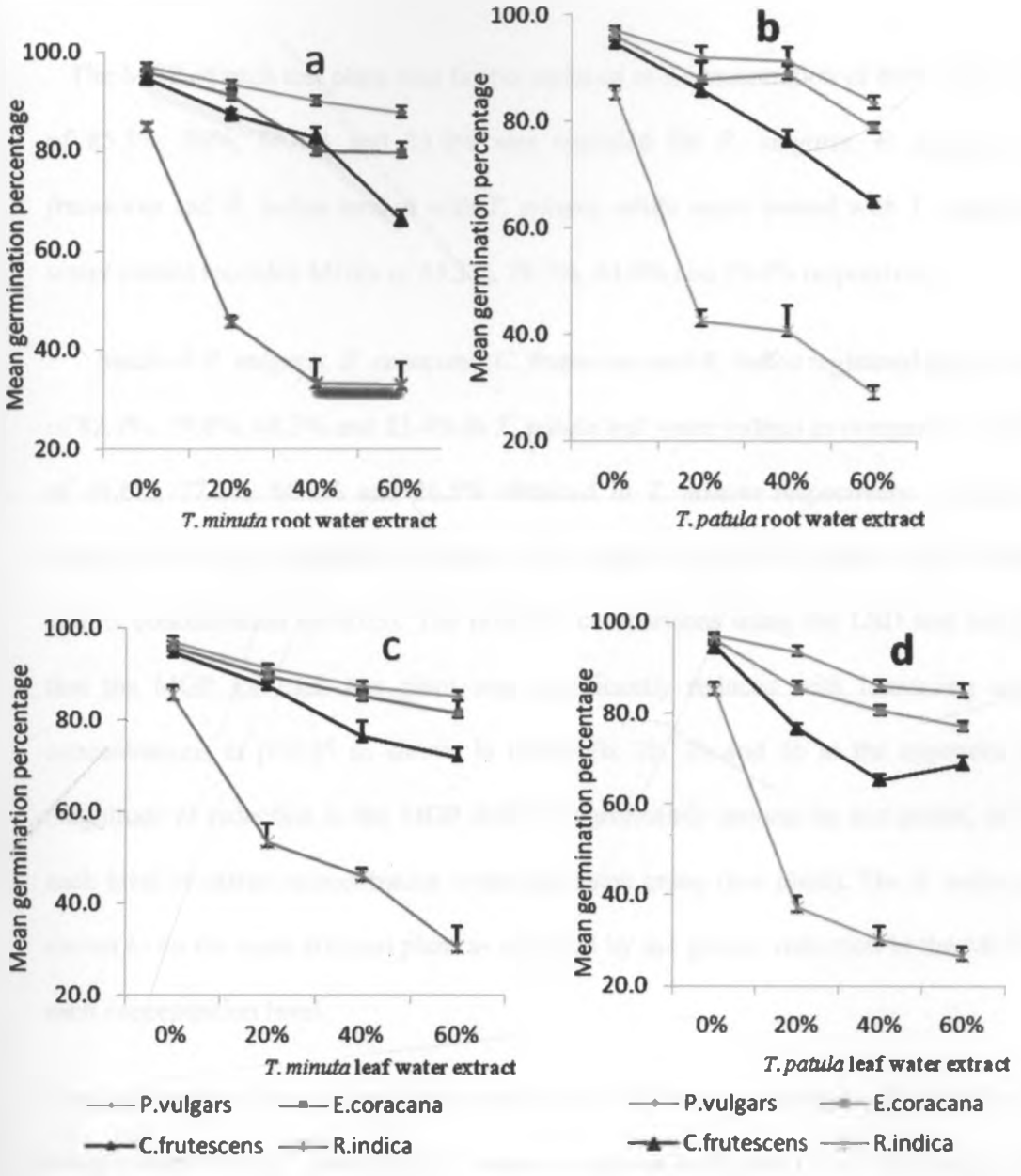


Figure 4.1 Effect of water extracts from (a & b) roots and (c & d) leaves of *T. minuta* and *T. patula* on the germination of *P. vulgaris, E. coracana, C. frutescens* and *R. indica* respectively. Values represent mean±SE at P = 0.05.

Comparatively, MGPs of 84%, 78.7%, 68.5% and 34.4% for *P. vulgaris, E. coracana, C. frutescens* and *R. indica* were recorded in water extracts from the leaves of *T. minuta*. Similarly, MGP values of 86%, 80.2%, 65.3% and 30.2% were obtained *P. vulgaris, E.*

coracana, C. frutescens and *R. indica* treated with water extracts from the leaves at a concentration of 40%.

The MGP of each test plant was further reduced at a concentration of 60%. The MGPs of 85.3%, 80%, 66.4% and 33.0% was recorded for *P. vulgaris, E. coracana, C. frutescens* and *R. indica* treated with *T. minuta,* while seeds treated with *T. patula* root water extract recorded MGPs of 83.3%, 78.7%, 64.8% and 29.0% respectively.

Seeds of *P. vulgaris, E. coracana, C. frutescens* and *R. indica* registered MGP values of 82.7%, 79.8%, 68.3% and 23.4% in *T. patula* leaf water extract as compared to MGPS of 84.6%, 77.1%, 68.8% and 26.5% obtained in *T. minuta* respectively. Analysis of variance showed a significant reduction in the MGP of each test plant with increasing extract concentration ($p<0.05$). The post hoc comparisons using the LSD test indicated that the MGP for each test plant was significantly reduced with increasing extract concentrations at $p<0.05$ as shown in tables 1b, 2b, 3b and 4b at the appendix. The magnitude of reduction in the MGP differed significantly among the test plants, and for each level of extract concentration within the same group (test plant). The *R. indica* was shown to be the most affected plant as reflected by the greater reduction in the MGP for each concentration level.

The germination time of each test plant was significantly prolonged ($P<0.05$) by the water extracts from *T. patula* and *T. minuta* as shown in figures (4.2 a, b, c and d). The mean germination periods in days (MGT) of 6.0, 4.2, 12.2 and 4.6 were recorded for *R. indica, E. coracana, C. frutescens* and *P. vulgaris* respectively in the control experiment. However, MGT for each test plant was prolonged, on the application of the extract. At a concentration of 20% MGPs of 6.8, 4.8, 12.4 and 5.2 days were registered for the seeds of *R. indica, E. coracana, C. frutescens* and *P. vulgaris* treted with *T. patula* root water

extract. The MGTs of 6.4, 5.0, 12.7, and 5.2 days recorded were recorded for *R. indica, E. coracana, C. frutescens* and *P. vulgaris* in *T. minuta* root water extract. Leaf water extracts at a concentration of 20% caused a further delay in the MGT of each test plant. In this case, *R. indica, E. coracana, C. frutescens* and *P. vulgaris* recorded an average period of 6.8, 5.4, 13.2, and 5.4 days in *T. minuta* leaf water extract, as compared to MGTs of 6.6, 5.4, 13.4 and 5.5 in days*T. Patula* leaf water extract respectively.

At a concentration of 40% the MGTs in days of 6.8, 5.4, 14.1 and 5.8 were recorded for *R. indica, E. coracana, C. frutescens* and *P. vulgaris* in *T. minuta* root water extract as compared to MGTs of 7.2, 6.0, 14.4 and 6.5 days respectively for the seeds treated with T. patula root water extract. Similar observations were made for the seeds treated with leaf water extracts at the same concentration. The MGT of 7.6, 6.5, 13.4 and 7.2 days were obtained for *R. indica, E. coracana, C. frutescens* and *P. vulgaris* respectively in *T. minuta* leaf water extract, while in *T. patula* leaf water extract at the same concentration 7.8, 6.3, 14.8 and 7.4 days were recorded respectively.

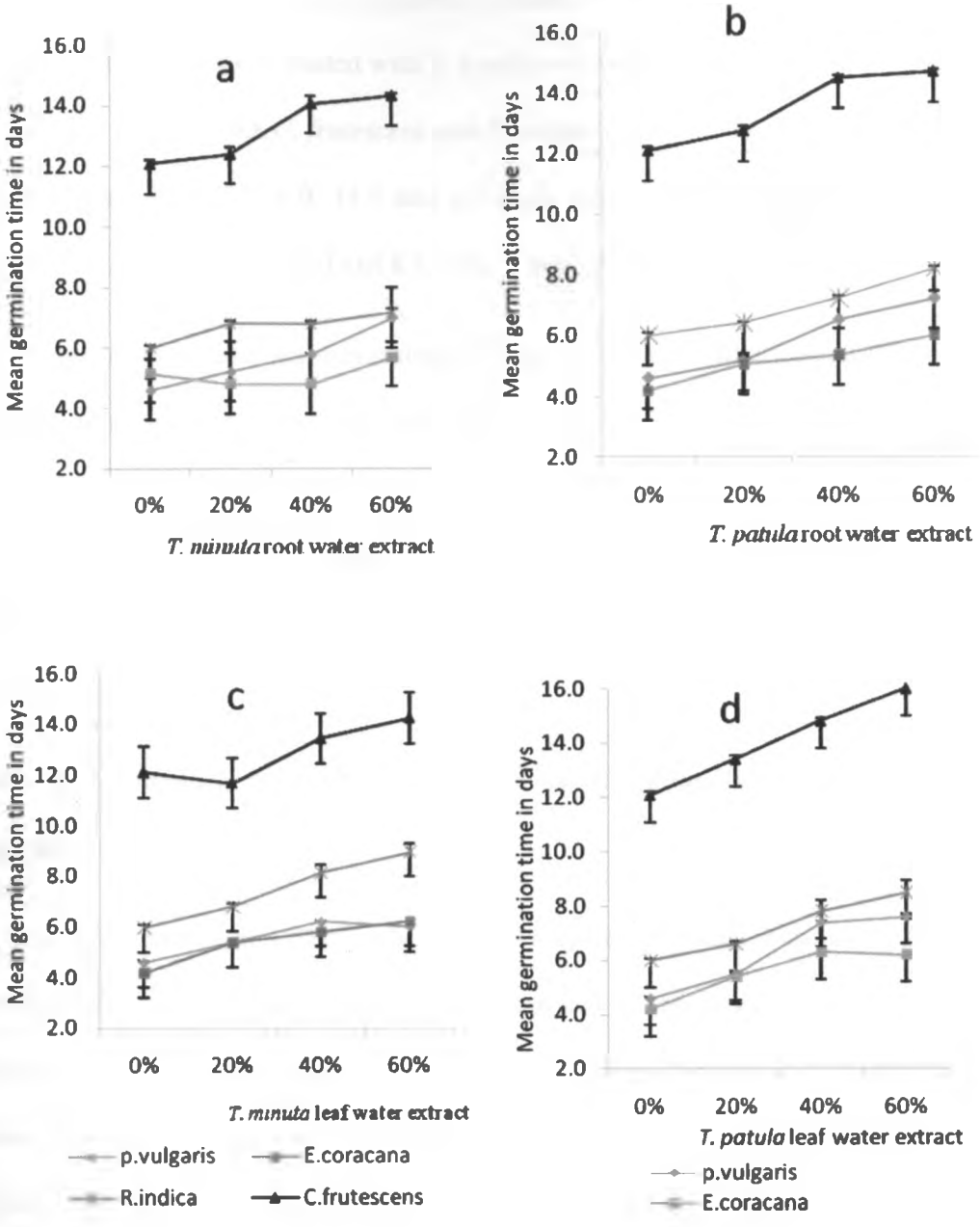


Figure 4.2 The influence of the water extracts from (a & b) roots and (c & d) leaves of *T. minuta* and *T. patula* on the mean germination time (mean±SE) for *P. vulgaris, E. coracana, C. frutescens* and *R. indica* at various concentrations ($P<0.05$).

The maximum concentration of 60% caused a significant delay in the germination of the test seeds. The MGT values of 7.0, 5.7, 14.4 and 7.2 days for *R. indica, E. coracana, C. frutescens* and *P. vulgaris* were observed on seeds treated with a concentration of 60%

T. minuta root water extract, while MGT values in days of 7.2, 7.0, 14.6 and 7.4 were recorded from the seeds treated with *T. patula* root water extract. . The observed MGT of *R. indica, E. coracana, C. frutescens* and *P. vulgaris* at a concentration of 60% *T. minuta* leaf extracts were 8.9, 6.0, 14.6 and 6.2 days, while seeds treated with *T. patula* leaf water extract registered MGTs of 8.5, 7.6, 16 and 7.8 days respectively.

It is shown statistically that increasing the water extract concentration causes a significant delay on the germination of the test plants at $p<0.05$. The post hoc test of comparison using the LSD test revealed that the mean germination time for the 0% concentration for each plant differed greatly from those of the higher concentrations. Significant delay at $p<0.05$ was noted at 60% concentration as indicated in tables 1e, 2e, 3e and 4e in the appendix 1.

4.2 The influence of methanol extracts of *T. minuta* and *T. patula* on seed germination.

Figures 4.3a, b, c and d show the effect of methanol extracts on the mean germination MGPs of *R. indica, C. frutescens, E. coracana* and *P. vulgaris*. The MGP reduction for each test plant was observed to be dependent on the concentration of the extract; however the magnitude of reduction differed among the test plants. The lowest MGP for each test plant was observed on the seeds of the test plants treated with 60% of the methanol extracts from the leaves compared to the roots. The MGPs of 43.0%, 80.7%, 65.2% and 83.9% were recorded for *R. indica, E. coracana C. frutescens* and *P. vulgaris* in *T. minuta* root methanol extract, while those treated with *T. patula* root methanol extract, registered MGPs of 41.2%, 83.0%, 72.5% and 89.4% respectively. Seeds treated with the *T. minuta* leaf extract at a concentration of 60%, recorded MGP of 36.3%, 79.5%, 68.9% and 85.3% for *R. indica, E. coracana C. frutescens* and *P. vulgaris*, while in *T. patula*

methanol extracts, MGP of the 42.3%, 81.5%, 71.0% and 86.2% were recorded respectively.

Higher values of MGPs were recorded for the seeds that were treated with methanol extract at a concentration of 40% as compared those of the seeds treated with 60% of the extract concentration. Seeds of *R. indica, E. coracana C. frutescens* and *P. vulgaris* treated with 40% of the methanol extract from the roots of *T. minuta* were observed to have the MGPs of 46.7%, 85.7%, 75.6% and 88.9% respectively, while the observed MGPs of the test plant species treated with the methanol extract from the roots of *T. patula* in the same order were 40.2%, 80.0%, 72.6% and 86%. Similarly, the methanol extracts from the leaves of *T. minuta* and *T. patula* were observed to have a reduction effect on the germination of the test plants. Seeds of *R. indica, E. coracana C. frutescens* and *P. vulgaris* treated with 40% of methanol extract from the leaves of *T. minuta* were observed to have the MGPs of 41.5%, 87.4%, 73.3% and 89.6%, while those seeds treated with 40% methanol extract from the leaves of T. *patula* leaves registered the MGP values of 34.1%, 80.7%, 76.3% and 86.7% respectively.

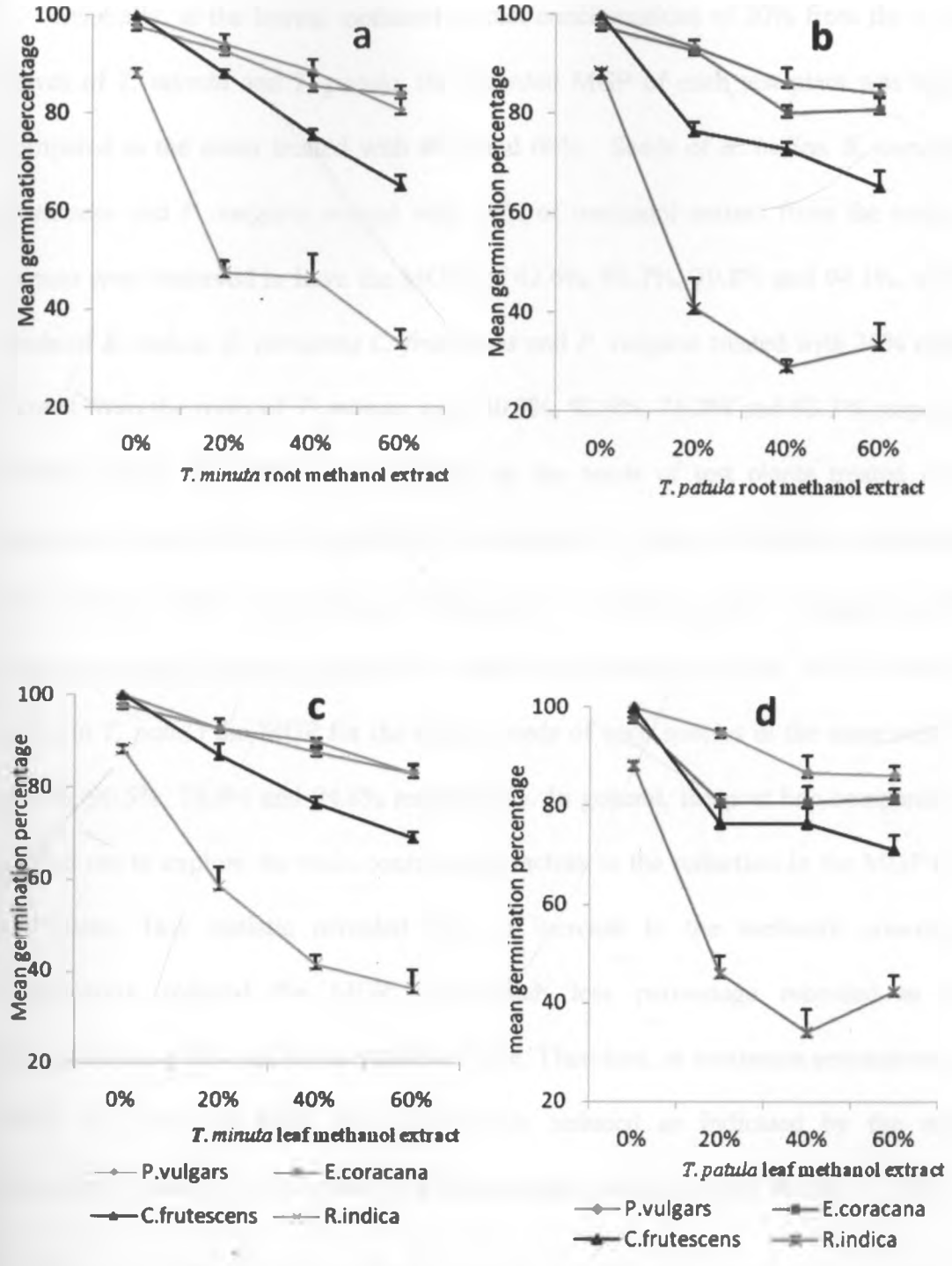


Figure 4.3 Effects of the methanol extracts from (a & b) roots and (c & d) leaves of *T. minuta* and *T. patula* on the germination of *P. vulgaris, E. coracana, C. frutescens* and *R. indica.* Results presented in the (Mean±SE) at $p<0.05$.

Generally, at the lowest methanol extract concentrations of 20% from the roots and leaves of *T. minuta* and *T. patula,* the recorded MGP of each test plant was higher as compared to the seeds treated with 40% and 60%. Seeds of *R. indica, E. coracana C. frutescens* and *P. vulgaris* treated with 20% of methanol extract from the roots of *T. minuta* were observed to have the MGPs of 47.6%, 92.7%, 79.8% and 94.1%, while the seeds of *R. indica, E. coracana C. frutescens* and *P. vulgaris* treated with 20% methanol extract from the roots of *T. minuta* were 40.7%, 92.6%, 76.3% and 93.3% respectively. Similar trends of results were observed on the seeds of test plants treated with the methanol extracts from the leaves of *T. minuta* and *T. patula* at the same concentration. The observed MGP of *R. indica, E. coracana C. frutescens* and *P. vulgaris* at 20% of methanol extract from the leaves of *T. minuta* were; 48.5%, 92.6%, 86.7% and 95.6%, while in *T. patula* the MGP for the treated seeds of each species in the same order were 46.0%, 90.5%, 79.8% and 94.8% respectively. In general, the post hoc comparison was carried out to explore the main contributing factors to the reduction in the MGP of each test plant. This statistic revealed that an increase in the methanol concentration significantly reduced the MGP with much less percentage recorded at higher concentrations (40% - 60%) as shown in table. Therefore, at maximum concentration, the MGP for each test plant was significantly reduced as indicated by the multiple comparison tables; 1c, 2c, 3c and 4c at the appendix, using the LSD test at $P = 0.05$.

The germination time of each test plant was statistically shown to be significantly delayed ($p<0.05$) with the increasing methanol extract concentration as shown in figures (4.4 a, b, c and d). Therefore, the response of each test plant seeds was shown to be dependent on the extract concentration, thus the higher the concentration, the longer the period the seeds took to germinate from the time of planting. The post hoc comparison

using the LSD test confirmed the significant delay for each test plant as observed in tables (1d & e, 2d & e, 3d and e and 4d & e) in appendix 1.

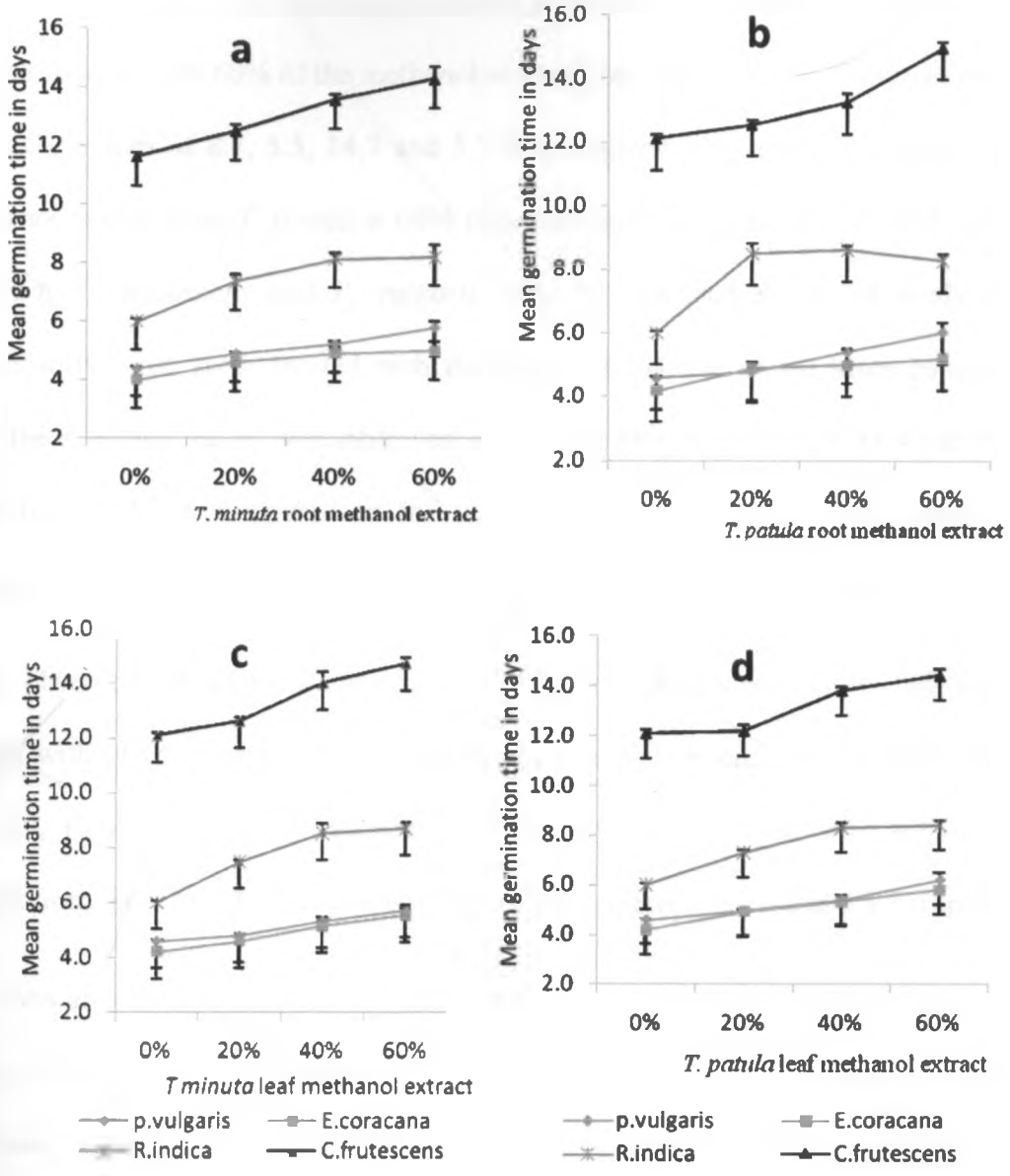


Figure 4.4 Effects of the methanol extracts from (a & b) root and (b & d) leaves of *T. minuta* and *T. patula* on the mean germination time (mean±SE) of *P. vulgaris, E. coracana, C. frutescens* and *R. indica.* ($p<0.05$).

In the case of methanol extract at 60% from the roots of *T. minuta*, the MGT of *R. indica, E. coracana C. frutescens* and *P. vulgaris* were 8.2, 5.0, 14.3 and 5.8 days

respectively. On the other hand, the MGT of *R. indica, E. coracana C. frutescens* and *P. vulgaris* treated with 60% methanol extract from the roots of *T. patula* were 8.3, 5.2, 14.9 and 6.0 days respectively. Similarly, seeds of *R. indica, E. coracana C. frutescens* and *P. vulgaris* treated with 60% of the methanol extract from the leaves of *T. minuta* registered the MGT values of 8.7, 5.5, 14.7 and 5.7 days respectively. It was also noted that the methanol extract from *T. patula* at 60% reduced significantly the MGTs of *R. indica, E. coracana C. frutescens* and *P. vulgaris* with the observed values of seeds reduced significantly those seeds treated with the methanol extracts of the same concentration from the *T. patula* leaves were observed to have the MGTs of 8.4, 5.8, 14.4 and 6.2 days respectively. When compared to the germination time of the seeds in the control treatment, the treated seeds were shown to take a longer period to germinate.

The observed MGTs for *R. indica, E. coracana C. frutescens* and *P. vulgaris* seeds treated with 40% methanol extract from the roots of *T. minuta* recorded the MGTs of 8.1, 4.9, 13.6 and 5.2 days respectively. Similarly, seeds treated with 40% methanol extract from the roots of *T. patula* registered MGTs of 8.6, 5.0, 13.2 and 5.4 respectively.

It was also evident that the MGT of test species treated with 40% methanol extract from the leaves were significantly prolonged ($p<0.05$). The test species; *R. indica, E. coracana C. frutescens* and *P. vulgaris* were observed to have the MGTs of 8.5, 5.1, 14.0 and 5.3 days respectively in 40% of methanol extract from the leaves of *T. minuta,* while seeds of *R. indica, E. coracana C. frutescens* and *P. vulgaris* treated with 40% methanol extract from the leaves of *T. patula* recorded the MGTs of 8.3, 5.3, 13.8 and 5.4 days respectively.

Seeds of *R. indica, E. coracana C. frutescens* and *P. vulgaris* treated with 20% of methanol extracts from the roots of *T. minuta* exhibited the MGTs of 6.8, 4.3, 12 and 4.7

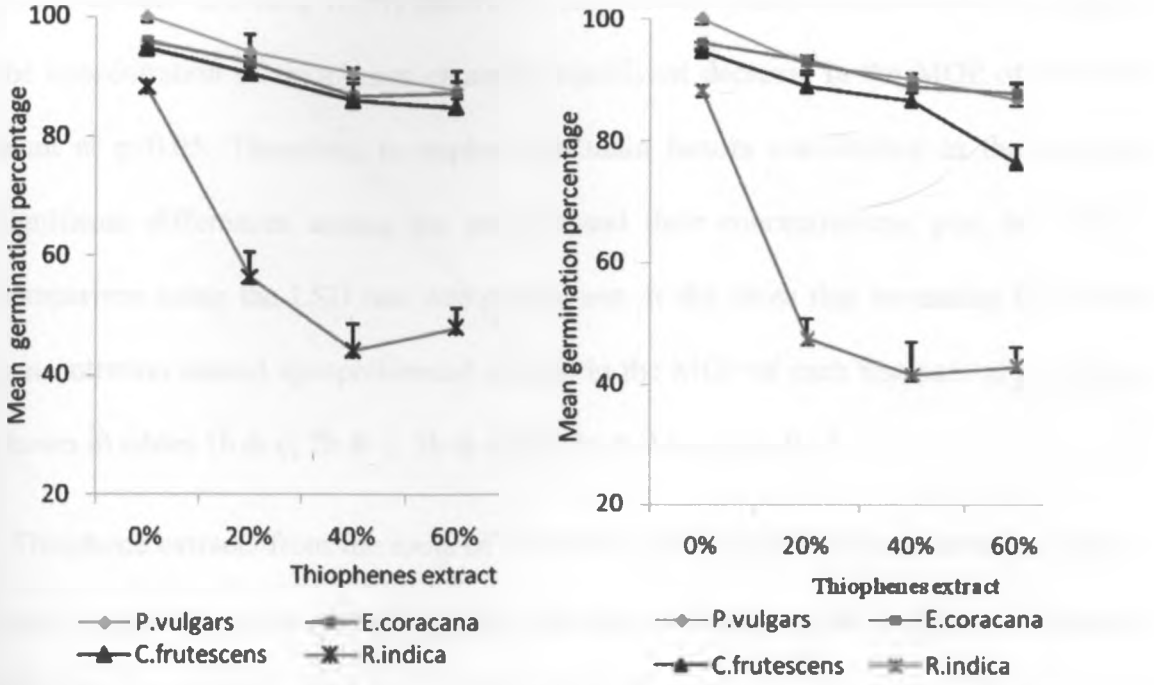
days respectively, while the MGT of each test species treated with 20% methanol from the roots of *T. patula* recorded the values of 7.7, 4.9, 12.5 and 4.8 days respectively. Similarly, the MGTs of *R. indica, E. coracana C. frutescens* and *P. vulgaris* treated with 20% methanol extracts from the leaves of *T. minuta* were 7.2, 4.8, 12.6 and 4.6 days, while those in *T. patula* were 7.3, 4.9, 12.2 and 5.0 days respectively.

There was a significant delay in the germination of the test plant seeds as the concentration of the extract was increased as shown in the post hoc tables; 1e, 2e, 3e and 4e in appendix 1, using the LSD test at P = 0.05. The magnitude of delay varied among the test plants as shown in figures 1.4 a, b, c and d.

4.3 Effects of Thiophenes extracts on seed germination.

The results on the germination responses of *E.coracana, P. vulgaris, C. frutescens* and *R. indica* seeds to thiophene extract from the roots of *T. minuta* and *T. patula* are shown in figures 4.5 a and b. The highest MGP for each test species was recorded in the control treatment with the values of 100%, 96%, 94.6% and 88.2% for *P. vulgaris, E. coracana, C. frutescens* and *R. indica* respectively. It was observed that the MGP of each test species significantly reduced by thiophene extract as shown in the post hoc tables (1b, 2b, 3b and 4b) of comparison using the LSD test at P = 0.05, in appendix 1.

Generally, the MGPs of *P. vulgaris, E. coracana, C. frutescens* and *R. indica seed* treated with 20g/100 ml (20%) of thiophene extract from *T. minuta* recorded the MGPs of 56.5%, 92.5%, 90.6% and 94.3% respectively, on the other hand, seeds of *P. vulgaris, E. coracana, C. frutescens* and *R. indica* treated with 20g/100 ml (20%) thiophene extract from the roots of *T. patula* recorded the MGPs of 47.4%, 93.2%, 88.8% and 92.5% respectively.



(a) *T. minuta* (b) *T. patula*

Figure 4.5 The influence of thiophene extracts from (a) *T. minuta* and (b) *T. patula* on the Mean germination percentages (mean±SE) for *P. vulgaris, E. coracana, C. frutescens* and *R. indica* seeds ($p<0.05$)

The MGP of each test species was observed to be reduced further on increasing the concentration of thiophenes to 40%. Seeds of *P. vulgaris, E. coracana, C. frutescens* and *R. indica* were observed to have the MGPs of 44.3%, 87.2%, 86.4% and 91.1% respectively, similarly, the seeds of test species treated with 40% thiophene extract from *T. patula* were observed to have the MGPs of 90.2%, 88.9%, 86.4% and 41.5%, respectively.

The germination percentage for each test plant reduced further at 60% concentration of thiophenes. MGPs of 88.2% 82.2%, 75.2% and 48.1%, were recorded for *P. vulgaris, E. coracana, C. frutescens* and *R. indica* seeds that were treated with 60% concentration of thiophenes from *T. minuta*. On the other hand, seeds of *P. vulgaris, E. coracana, C. frutescens* and *R. indica* treated with 60% of thiophenes extract of *T. patula* recorded the

MGPs of 86.7%, 87.8%, 76.3% and 42.9% respectively. Statistics showed that increasing the concentration of thiophenes caused a significant decrease in the MGP of each test plant at $p<0.05$. Therefore, to explore the main factors contributing to the observed significant differences among the extracts and their concentrations, post hoc test of comparison using the LSD test was carried out. It did show that increasing the extract concentration caused apropotionated decline in the MGP of each test pant at $p<0.05$, as shown in tables 1b & c; 2b & c; 3b & c; and 4c & d in appendix 1.

Thiophene extracts from the roots of *T. minuta* and *T. patula* were observed to cause a significant delay on the germination of each test species as shown in figures 4.6a and c. The delay was observed to be proportional to the extract concentration. The recorded MGTs in the control treatment were 4.8, 4.3, 12.2 and 6.4 for *P. vulgaris, E. coracana, C. frutescens* and *R. indica* respectively.

Seeds of *P. vulgaris, E. coracana, C. frutescens* and *R. indica* subjected to a concentration of 20% thiophenes extract from the roots of *T. minuta,* recorded the MGT values of 4.9, 4.7, 12.8 and 7.2 days respectively, while in *T. patula* thiophenes extract gave the values of 5.0, 4.8, 13.1 and 7.3 days respectively. This indicated a slight delay in germination of the test plant seeds.

Increasing the concentration of thiophenes to 40% caused a significant delay on the MGT of each test species compared to the control treatment. Seeds of *P. vulgaris, E. coracana, C. frutescens* and *R. indica* treated with 40% *T. minuta* thiophene extract, registered the MGTs of 5.6, 5.2, 13.3 and 8.2 days respectively, while those seeds treated with thiophene extract from the roots of *T. patula* of the same concentration were observed to have the MGTs of 5.5, 5.0, 13.6 and 8.4 days respectively.

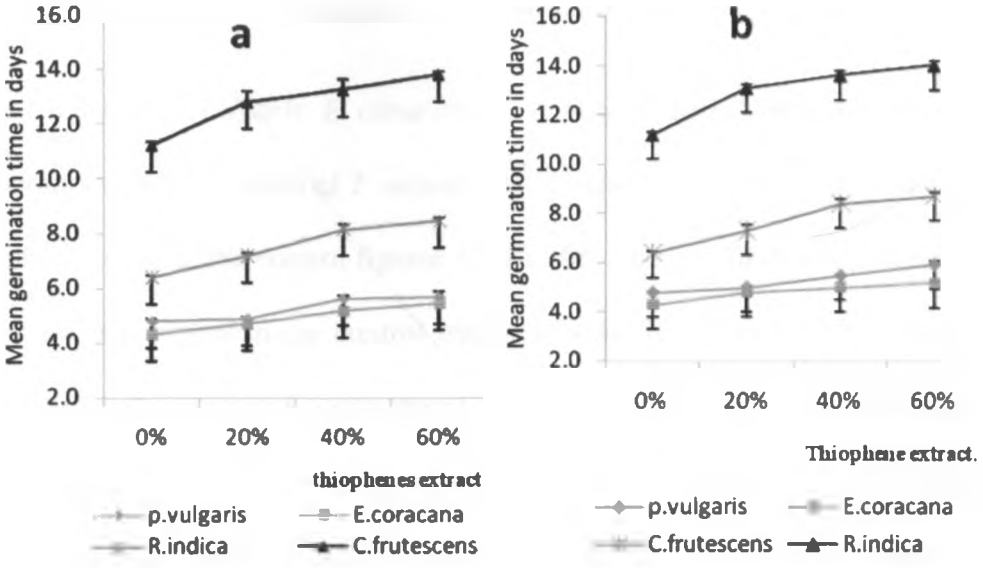


Figure 4.6 Influence of thiophenes extract from (a) roots of *T. minuta* and (b) *T. patula* on the germination time of the test plants. The values presented in mean±SE.

The MGT of each test species was observed to be further prolonged on increasing the concentration of thiophenes to 60%. The MGT values of 5.7, 5.5, 13.8 and 8.5 were recorded for *P. vulgaris, E. coracana, C. frutescens* and *R. indica* seeds treated with 60% of thiophenes extract from *T. minuta*. Seeds of the test plants in that order, gave the MGT values of 5.9, 5.2, 14.0 and 8.7 days in thiophenes extract from *T. patula*. The germination reduction was observed to be propotional with the increase in thiophene extract concentration. Analysis of various revealed that thiophenes caused a significant reduction in the germination of the test plant seeds. The MGP was shown to be significant at $p<0.05$ by the post hoc comparison using the LSD test as the concentration of thiophene increased as shown in tables 1d & e; 2d & e; 3d & e; and 4d & e in appendix 1.

4.4 Effects of residues of *T. minuta* and *T. patula* on seed germination.

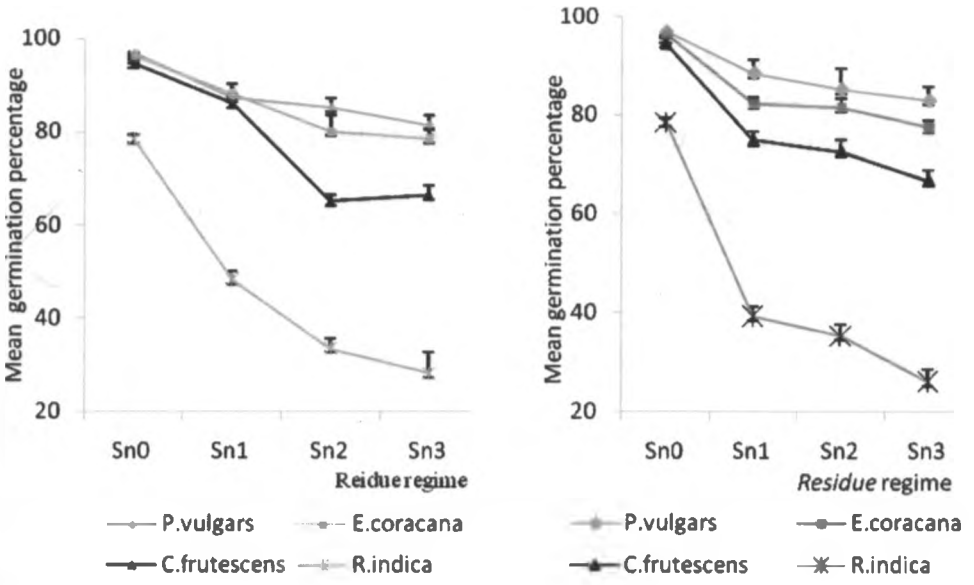
The MGPs of *P. vulgaris, E. coracana, C. frutescens* and *R. indica* seeds sown in soil incorporated with the residue of *T. minuta* and *T. patula* were observed to be significantly reduced compared to the control figures 4.7 a and b. The germination percentage for each test species was higher in the control treatment with the values of 97%, 96.4%, 94.5% and 78.5% for *P. vulgaris, E. coracana, C. frutescens* and *R. indica.* Analysis of variance indicated that the residue regimes from *T. minuta* and *T. patula* significantly reduced the germination percentage of each test plant, though the magnitude of reduction varied among the test species. It was also observed that the level of MGP reduction for each test plant was dependent on the level of the residue regime, with the lowest MGP recorded on the regime three. This was confirmed by the post hoc test of comparison using the LSD test, which indicated that at higher residual regime (Sn3), the MGP of each test plant was significantly reduced at $p<0.05$ as shown in tables 1b & c; 2b & c; 3b & c; and 4c & d in appendix 1.

Seeds of *P. vulgaris, E. coracana, C. frutescens* and *R. indica* sown in soils with the residue of *T. minuta* at regime one were observed to have the MGPs of 87.4%, 88.1%, 85.9% and 48.1% respectively, while those seeds of test species sown in the soil incorporated with residue of *T. patula* recorded the MGPs of 88.5%, 82.2%, 74.8% and 39.2% respectively.

The MGPs of each test plant was observed to be significantly reduced on the seeds sown in soils containing residue of the second regime from *T. minuta* and *T. patula*. The observed MGPs of *P. vulgaris, E. coracana, C. frutescens* and *R. indica* at the second regime of the residue from *T. minuta* were 85.2%, 80.0%, 65.0% and 33.5% respectively. Similarly, seeds of *P. vulgaris, E. coracana, C. frutescens* and *R. indica*

sown in soils incorporated with the residue of the second regime from *T. patula* recorded MGPs of 85.2%, 81.5%, 72.6% and 35.2% respectively.

Highest level of germination reduction for each test species was observed on the seeds planted in the soils with the residue of regime from *T. minuta* and *T. patula.* The observed MGPs of *P. vulgaris, E. coracana, C. frutescens* and *R. indica* sown in soils with residue of regime three from *T. minuta* were 81.5%, 78.5%, 66.4% and 28.1% respectively. Similarly, the seeds of *P. vulgaris, E. coracana, C. frutescens* and *R. indica* sown in soils incorporated with residue of the third regime from *T. patula* were observed to have the MGP values of 83.0%, 77.5%, 66.6% and 26.0%



(a) *T. minuta* (b) *T. patula*

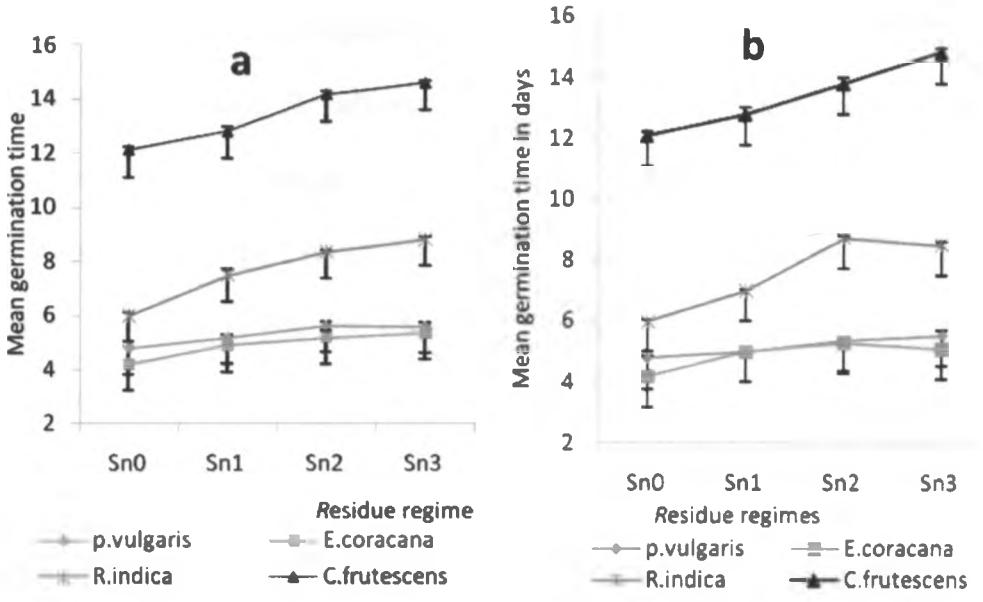
Figure 4.7 The effect of 14 day seedling residues from (a) *T. minuta* and (b) *T. patula* on the germination of *P. vulgaris, E. coracana, C. frutescens* and *R. indica.* Values presented in mean±SE

Similarly, the residue significantly prolonged the germination time of each test plant as shown in figures 4.8a and c. The lowest MGT values were recorded in the control treatment with *P. vulgaris, E. coracana, C. frutescens* and *R. indica* having 4.8, 5.2, 12.1

and 6.0 days respectively. Seeds of *P. vulgaris, E. coracana, C. frutescens* and *R. indica* treated with residue of regime one from *T. minuta*, recorded the MGT of 5.2, 4.9, 12.8, and 7.5 days respectively, while those seeds sown in the residue of *T. patula* at the same regime recorded the MGTs of 5.0, 5.0, 12.8 and 7.0 days respectively.

Generally, the MGTs of *P. vulgaris, E. coracana, C. frutescens* and *R. indica* seeds were significantly prolonged by the residue of the second regime from *T. minuta* with the values of 5.7, 5.2, 14.2 and 8.4 days respectively, while those seeds treated with the residue of the second regime of *T. patula* recorded the MGTs of 5.4, 5.3, 13.8 and 8.7 days respectively.

The MGT of each test plant was observed to decline further on the seeds treated with residue of regime (Sn3). Seeds of *P. vulgaris, E. coracana, C. frutescens* and *R. indica* sown in soil containing residue of the third regime recorded the MGTs of 5.6, 5.4, 14.6 and 8.8 days respectively. Similarly, MGTs of 5.5, 5.1, 14.8 and 8.5 days were obtained for *P. vulgaris, E. coracana, C. frutescens* and *R. indica* seeds sown in soils incorporated with Sn3 of *T. patula.* It was shown statistically by the post hoc test of comparison using the LSD at $p<0.05$ that the MGT each test plant delayed with the increase in the residual regime and that the length of delay varied with the type of the test species as indicated in tables 1d & e; 2d & e; 3d & e; and 4d & e in appendix 1.



(a) *T. minuta* (b) *T. patula*

Figure 4.8 The effects of 14 day seedling residues of (a) *T. minuta* and (b) *T. patula* on the germination time of seeds. Values represent the mean±SE.

The results indicated that *R. indica* was the most inhibited species followed by *C. frutescens* and *E. coracana* with *P. vulgaris* the least affected species by all extract treatment from *T. minuta* and *T. patula* as shown in figures1.8. Similarly, the highest level of extract concentration was observed to cause the highest germination inhibition effect. On the same note, the various extracts from *T. minuta* and *T. patula* were observed to reduce the mean germination to different levels.

4.5 Effects of water extract from *T. minuta* and *T. patula* on seedling growth.

The allelochemical influence of the aqueous extract on seedling growth was illustrated by comparing the root length (RL), shoot length (SL) and leaf area (LA) of the treated seedlings as shown in figure (4.9ba &b; 4.2.1 a & b). In the control treatment, seedlings of *E.coracana, P. vulgaris, C. frutescens* and *R. indica* recorded the average root lengths

of 3.1, 5.0, 2.2 and 3.4 cm respectively. The RL values of 3.0, 5.3, 1.8 and 2.8 cm were recorded for *E.*coracana, *P. vulgaris, C. frutescens* and *R. indica* seedlings treated with *T.minuta* root water extract at a concentration of 20%, while SL values of 2.6, 6.1, 2.2 and 3.7 cm and with the LAs of 0.4, 14.7, 0.3 and 0.5 cm^2 were recorded respectively. Similarly, RL values of 2.8, 4.8, 1.6 and 2.7 cm, while the SL of 2.3, 5.9, 2.5 and 3.2 cm and LAs of 0.4, 14.2, 0.2 and 0.5 cm^2 for treated with 20% water extract from the roots of *T. patula* respectively.

Water extract from the leaves *T. minuta* and *T. patula* significantly reduced the RL and SL of the E.*coracana, P. vulgaris, C. frutescens* and *R. indica* seedlings with RLs of 2.3, 4.8, 1.7 and 2.3 cm, with SLs of 2.6, 5.7, 2.4 and 3.4 cm, while the leaf area was non significantly affected by the water extract, though there was a slight reduction as shown in figure 4.2.1 a and b.

The RL, SL and LA of the test plants were further reduced on the seedlings treated with 40% water extract; however the magnitude of reduction for each parameter varied for each test species. Seedlings of *E.coracana, P. vulgaris, C. frutescens* and *R. indica* that were treated with 40% (w/v) *T. minuta* water extract registered, RL values of 2.6, 4.9, 1.2, 2.4 cm; SLs values of 3.0, 5.6, 2.1, 2.3 cm and LAs of 0.4, 11.6, 0.3, 0.3 cm^2 respectively. Seedlings of *E.coracana, P. vulgaris, C. frutescens* and *R. indica* that were subjected to 40% *T. patula* root water extract, recorded RL values of 2.6, 4.3, 1.5, 2.6 cm; SL values of 2.6, 5.3, 2.1, 2.9 cm and LA values of 0.3, 12.6, 0.3, 0.4 cm^2.

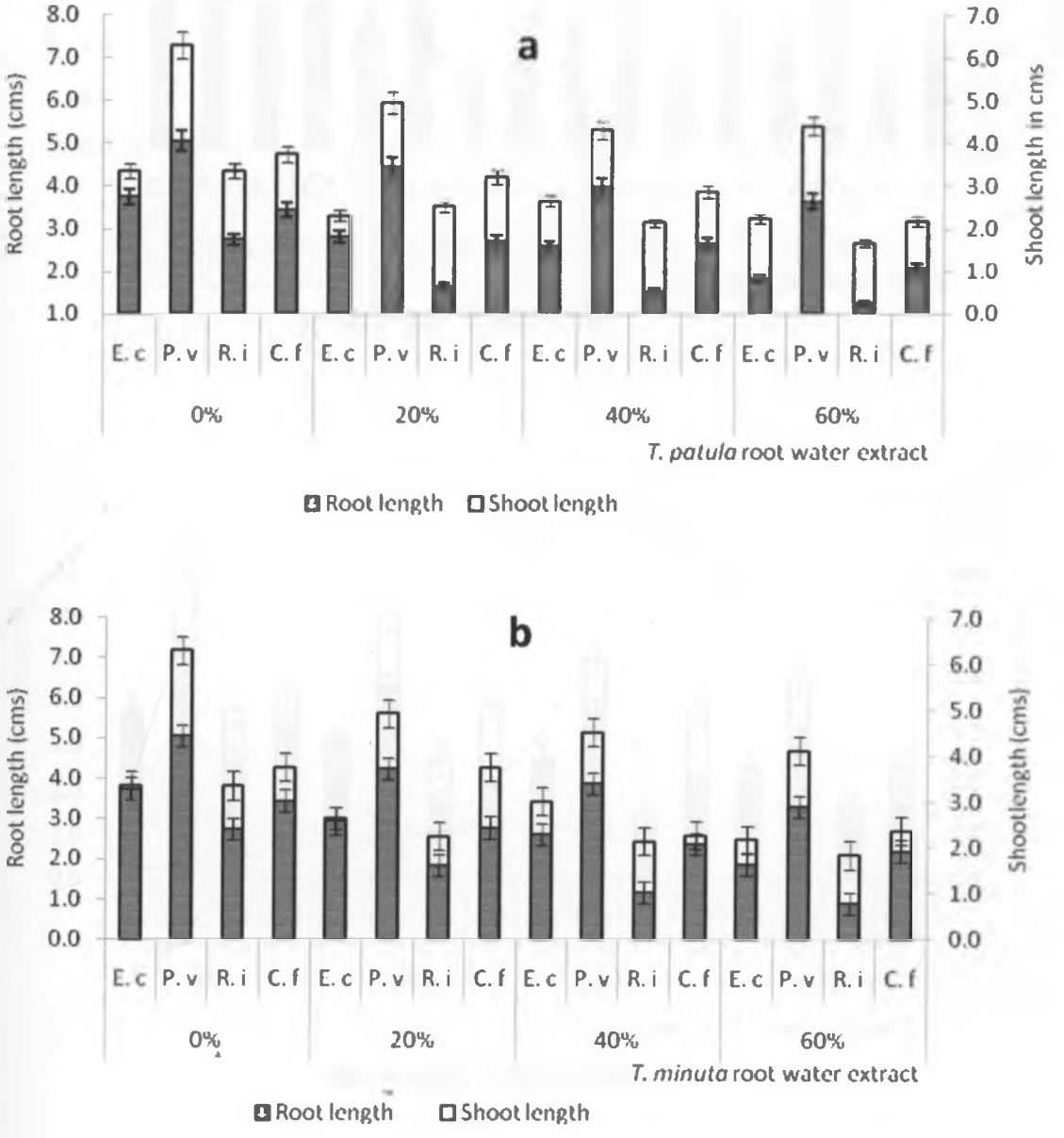
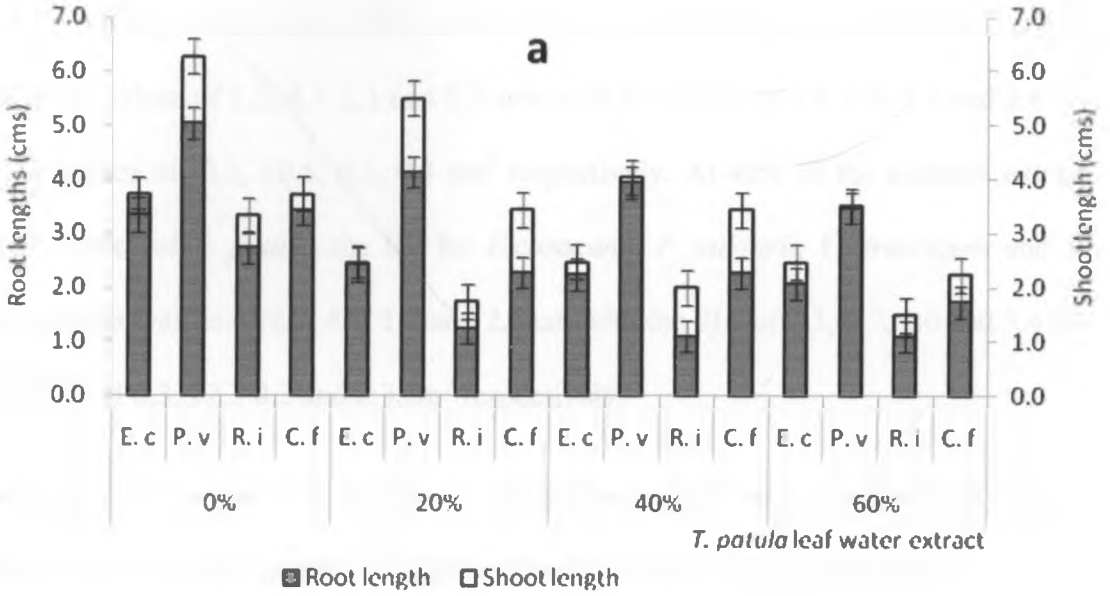


Figure 4.9 The effects of water extracts from the roots of (a) *T. patula* and (b) *T. minuta* on the shoot and root lengths of *E. coracana, p. vulgaris, C. frutescens* and *R. indica* seedlings. Values presented in mean±SE; ($p<0.05$).



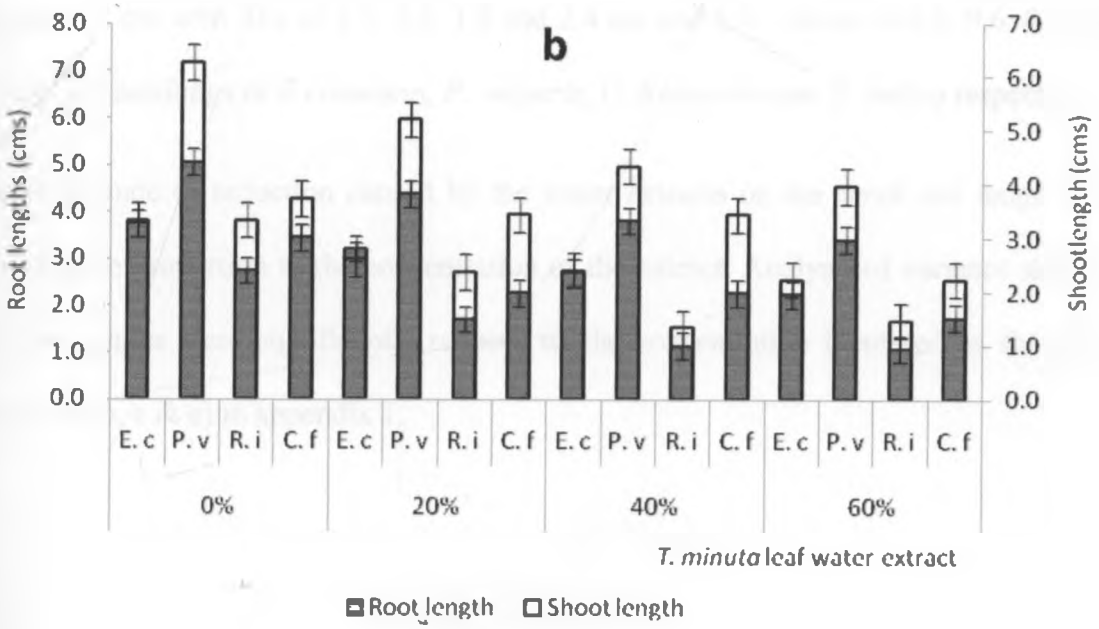


Figure 4.2.1 The effects of the water extract from the leaves of (a)*T. patula* and (b) *T. minuta* on the shoot and root lengths of *E. coracana, p. vulgaris, C. frutescens* and *R. indica* seedlings. Values represent the mean±SE; ($p<0.05$).

The response of the seedlings to 40% of the aqueous extract from the leaves of *T. minuta* and *T. patula* reduced significantly the LA, SL and RL as compared to the control

treatment. The observed values for the LA of *E.coracana, P. vulgaris, C. frutescens* and *R. indica* seedlings treated with 40% of the aqueous extract from the leaves of *T. minuta* recorded RL values of 2.2, 4.5, 1.1 and 2.5 cms with SL values of 2.4, 5.4, 1.3 and 3.4 cm and LAs values of 0.3, 10.5, 0.3, 0.3 cm^2 respectively. At 40% of the aqueous extract from the leaves of *T. patula*, the RL for *E.coracana, P. vulgaris, C. frutescens* and *R. indica* recorded values of 2.0, 4.7, 1.1 and 2.3 cm with the SLs of 2.5, 5.7, 2.0 and 3.4 cm and the LAs of 0.3, 12.3 0.2 and 0.3 cm^2 respectively.

Seedlings of *E.coracana, P. vulgaris, C. frutescens* and *R. indica* treated with 60% of aqueous extract from the roots of *T. minuta* were observed to have the values of 1.8, 4.4, 0.8 and 2.2 cm for the RLs with SL were 2.2, 5.5, 1.8 and 2.4 cm also the LA values of 0.3, 9.6 0.3 and 0.4 cm^2 respectively, while in *T. patula* extract the RL values of 2.0, 4.4, 0.9 and 2.2 cm with SLs of 2.2, 5.5, 1.8 and 2.4 cm and LA values of 0.3, 9.6, 0.3 and 0.3 cm^2 for seedlings of *E.coracana, P. vulgaris, C. frutescens* and *R. indica* respectively.

The magnitude of reduction caused by the water extracts on the shoot and length was shown to be proportion to the concentration of the extract. Analysis of variance showed that the lengths were significantly reduced as the concentration increased as shown in tables (5a, b, c & e) in appendix 1.

Table 4. 1 Effect of various water extract concentrations from *T. minuta* and *T. patula* to leaf area presented in mean±SE of *E. coracana, P. vulgaris, C. frutescens* and *R. indica*

		T. p Rw	T. m Rw	T.p Lw	T. mLw
Concentration	testplant	Leaf area	Leaf area	Leaf area	Leaf area
0% (control)	*E. coracana*	0.40 ±0.01	0.41 ±0.02	0.39 ±0.01	0.39 ±0.01
	P .vulgaris	15.77±0.63	15.01±0.02	16.02±0.02	14.05±0.03
	R. indica	0.32±0.02	0.33±0.01	0.30±0.02	0.31±0.01
	C. frutescens	0.54±0.01	0.55±0.02	0.53±0.01	0.53±0.01
20%(w/v)	*E. coracana*	0.39±0.01	0.38±0.02	0.32±0.02	0.36±0.02
	P .vulgaris	14.23±0.26	14.73±0.18	13.83±0.23	15.07±0.38
	R. indica	0.25±0.01	0.27±0.01	0.23±0.01	0.27±0.01
	C. frutescens	0.49±0.05	0.53±0.01	0.28±0.01	0.28±0.01
40%(w/v)	*E. coracana*	0.33±0.00	0.36±0.02	0.33±0.01	0.32±0.01
	P .vulgaris	12.63±0.41	11.63±0.30	12.30±0.26	13.27±0.23
	R. indica	0.30±0.01	0.26±0.02	0.23±0.03	0.19±0.01
	C. frutescens	0.35±0.02	0.34±0.02	0.28±0.01	0.28±0.01
60%(w/v)	*E. coracana*	0.29±0.03	0.30±0.01	0.30±0.02	0.30±0.02
	P .vulgaris	7.83±0.38	9.60±0.32	10.67±0.26	10.73±0.20
	R. indica	0.24±0.01	0.27±0.01	0.15±0.01	0.19±0.01
	C. frutescens	0.31±0.01	0.35±0.01	0.18±0.02	0.20±0.02

Key

T. p Rw *Tagetes patula* root water extract T. m Rw *Tagetes minuta* root water extract

T.p Lw *Tagetes patula* leaf water extract T. mLw *Tagetes minuta* leaf water extract

(w/v) weight per volume (grams/litre)

4.6 The influence of methanol extracts of *T. minuta* and *T. patula* on seedling growth.

Methanol extracts from the leaves and roots of *T. minuta* and *T. patula* were observed to have a reductive effect on the development of the leaf area, root and shoot length of *E.coracana, P. vulgaris, C. frutescens* and *R. indica* seedlings as shown in figures 4.2.2 a & b; 4.2.3 a & b. The measureable parameters in the control treatment recorded the highest values. In this case, the LA values of 0.42, 14.4, 0.46 and 0.36 cm^2 were recorded for *P. vulgaris, C. frutescens* and *R. indica* seedlings respectively. Similarly, RLvalues of

3.23, 5.03, 3.23 and 2.6 cm and SL values of 3.8, 6.0, 4.07 and 3.2 cm were recorded respectively.

Seedlings of *E.coracana, P. vulgaris, C. frutescens* and *R. indica* that were established in a concentration of 20% *T. minuta* methanol extract, recorded the mean values of 2.9, 4.5, 2.8 and 2.1 cm for the RLs respectively, while 3.3, 5.4, 3.3 and 2.6 cm were recorded for SL respectively and,0.4, 13.8, 0.4 and 0.3 cm^2 for the LA. On the other hand, RL values of 2.6, 4.3, 2.8 and 1.8 cm with SL values of 3.0, 5.7, 2. 9 and 2.3 cm and LA values of 0.3, 13.9 0.3 and 0.3 cm^2 for *E.coracana, P. vulgaris, C. frutescens* and *R. indica* seedlings that were treated with 20% extract concentration of *T. patula* root methanol extract. Similarly, 20% of methanol extract from the leaves of *T. minuta* significantly reduced seedling growth with the RL values of 2.5, 5.8, 2.5 and 2.0 cm with the SLs of 2.9, 6.4, 3.7 and 2.4 cm and the LAs of 0.5, 14.2, 0.4 and 0.3cm^2 for *E.coracana, P. vulgaris, C. frutescens* and *R. indica* respectively. On the other hand RL values of 2.3, 5.4, 2.4 and 1.8 cm were recorded for *E.coracana, P. vulgaris, C. frutescens* and *R. indica* seedlings treated with 20% of methanol extract from the leaves of *T. patula.* In the same seedlings the SLs of 2.8, 6.0, 3.5 and 2.2 cms with the LAs of 0.4, 13.9, 0.2 and 0.2 cm^2 were recorded.

Seedlings of *E.coracana, P. vulgaris, C. frutescens* and *R. indica* treated with 40% of the methanol extract from the roots of *T. minuta* recorded much lower values for the RL, SL and LA. The RL values of 2.2, 4.1, 2.3 and 1.3 cm, with SL values of 2.6, 5.4, 3.3 and 2.1 cm with LA values of 0.3, 13.0, 0.3 and 0.2 cm^2 were recorded for *E.coracana, P. vulgaris, C. frutescens* and *R. indica* seedlings respectively. Seedling also subjected to 40% of the methanol extract from the roots of *T. patula* were observed to have the mean values for RLs of 2.0, 3.8, 2.1 and 1.2 cm with the SLs of 2.6, 5.0, 3.3 and 2.1 and the

LAs of 0.3, 12.1, 0.3 and 0.3 cm^2 for *E.coracana, P. vulgaris, C. frutescens* and *R. indica* respectively.

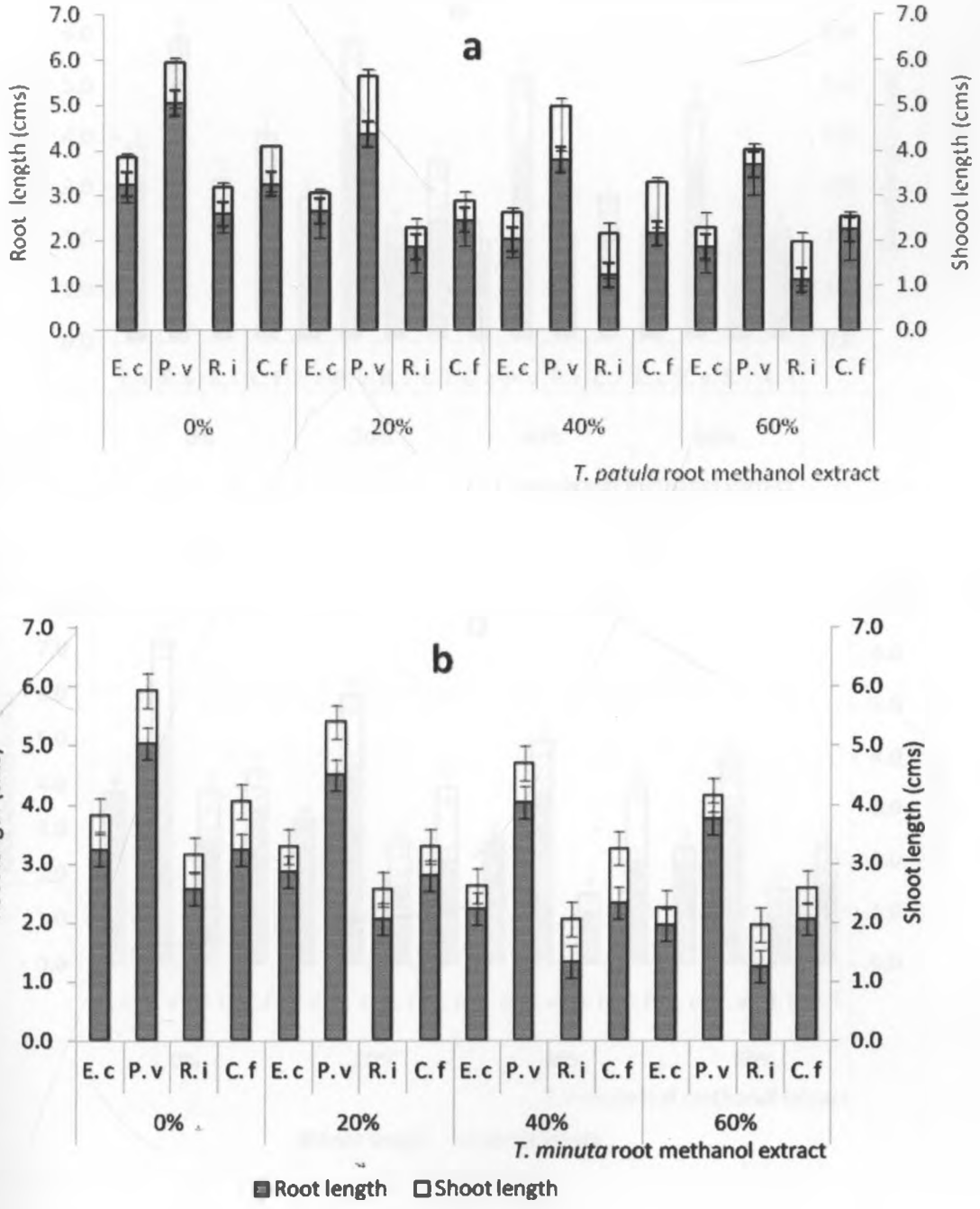


Figure 4.2.2 The influence of the methanol extract from the roots of (a) *T. patula* and (b) *T. minuta* on the shoot length and root length of *E. coracana, p. vulgaris, C. frutescens* and *R. indica* seedlings. Values represent the mean±SE; ($p<0.05$)

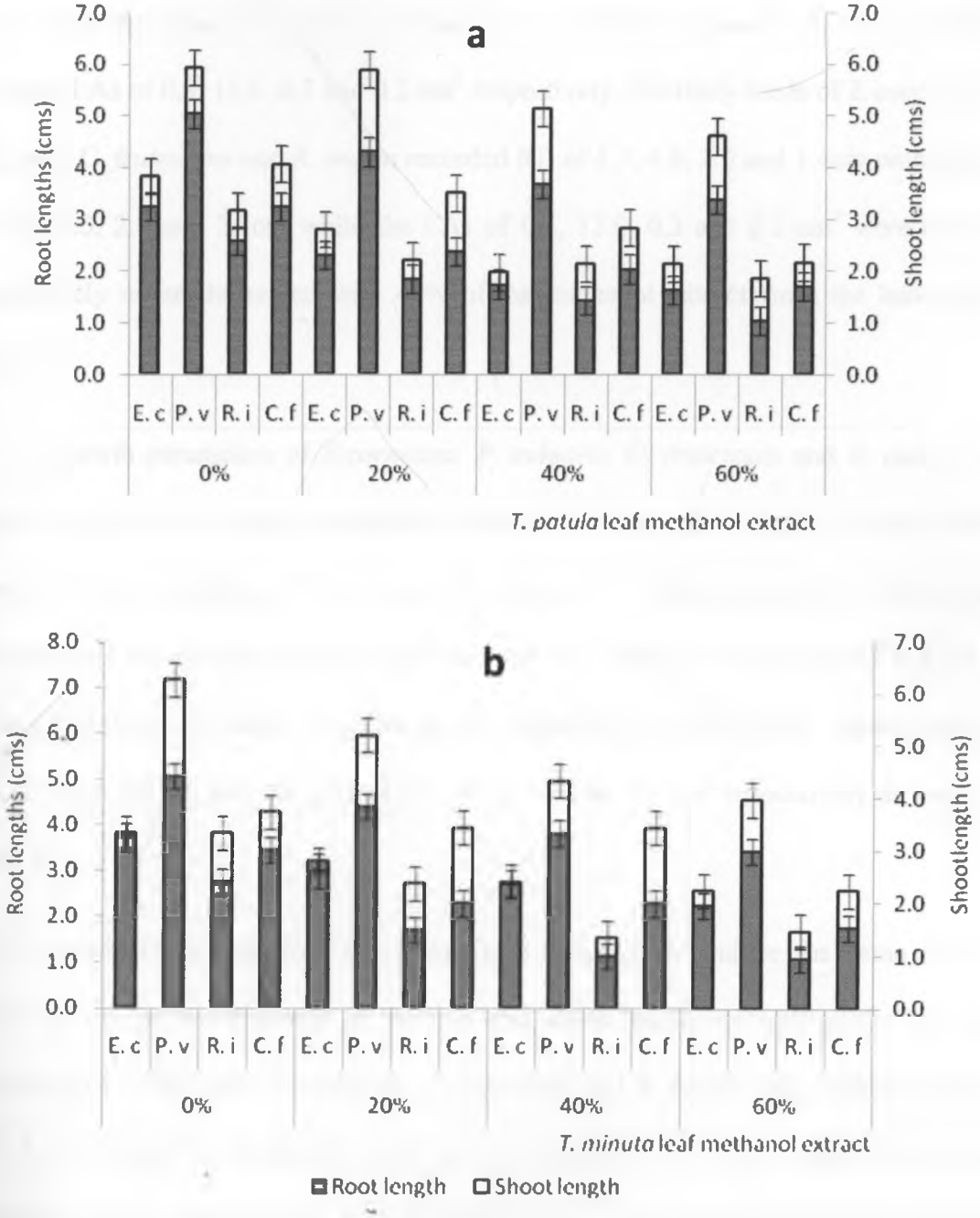


Figure 4.2.3 The influence of the methanol extracts from the leaves of (c) *T. patula* and (d) *T. minuta* on the shoot length and root length of *E. coracana, p. vulgaris, C. frutescens* and *R. indica* seedlings. Values represent the mean±SE; ($p<0.05$)

The response of *E.coracana, P. vulgaris, C. frutescens* and *R. indica* seedlings treated with 40% methanol extract from the leaves of *T. minuta*, showed a decline in RLs which recorded values of 1.9, 4.6, 2.0 and 1.6 cm, while the SLs of 2.2, 5.7, 3.2 and 2.1 cm with LAs of 0.3, 12.6, 0.3 and 0.2 cm^2 respectively. Similarly seeds of *E.coracana, P. vulgaris, C. frutescens* and *R. indica* recorded RL of 1.7, 4.9, 2.0 and 1.4cm with the SLs of 2.0, 5.5, 2.8 and 2.1cm while the LAs of 0.3, 12.0, 0.3 and 0.2 cm^2 were recorded respectively on seeds treated with 40% of the methanol extract from the leaves of *T. patula.*

The growth parameters of *E.coracana, P. vulgaris, C. frutescens* and *R. indica* were highly reduced on the seeds treated with 60% of methanol extract from *T. minuta* and *T. patula*. Treated seedlings of *E.coracana, P. vulgaris, C. frutescens* and *R. indica* treated with 60% of the methanol extract from the roots of *T. minuta* were observed to have the values of 2.0, 4.2, 2.1 and 1.3 cm for the RL respectively, while the SL values were 2.3, 5.0, 2.6 and 2.0 cm and the LAs of 0.3, 11.6, 0.3 and 0.2 cm^2 respectively as shown in table 4.3.

The methanol extract from the leaves was observed to induce the same trend of reduction on the development of the root and shoot lengths as well as the leaf area. Seedlings of *E.coracana, P. vulgaris, C. frutescens* and *R. indica* were observed to have 1.3, 4.6, 2.0 and 1.3 cm for RL while the SL values of 1.8, 4.9, 2.3 and 2.0 cm were recorded and LA values of 0.3, 10.5, 0.4 and 0.3 cm^2 were observed on treating the seeds with 60% of methanol extract from the leaves of *T. minuta.* Similar trend of reduction were observed on the RLs, SLs and LAs of the seedlings treated with 60% of methanol extract from the roots of *T. patula.* Analysis of various showed that the methanol extracts

significantly reduced the SL and RL at $p<0.05$, however the LA was non significantly affected by the methanol extracts as shown in tables 5a, b & e in appendix 1.

Table 4.2 Effects of methanol extracts of *T. minuta* and *T. patula* on the leaf area (mean±SE) of *E. coracana, P. vulgaris, C. frutescens* and *R. indica*

		T. p Rm	T. m Rm	T.p Lm	T. mLm
Concentration		Leaf area	Leaf area	Leaf area	Leaf area
0% (control)	*E. coracana*	0.42±0.02	0.43±0.01	0.41±0.03	0.43±0.01
	P .vulgaris	14.40±0.12	15.10±0.10	14.20±0.10	15.30±0.15
	R. indica	0.36±0.01	0.38±0.02	0.35±0.02	0.37±0.02
	C. frutescens	0.46±0.01	0.47±0.03	0.45±0.02	0.45±0.02
20% (w/v)	*E. coracana*	0.33±0.01	0.35±0.02	0.40±0.01	0.45±0.01
	P .vulgaris	13.93±0.26	13.80±0.35	13.87±0.18	14.17±0.15
	R. indica	0.28±0.02	0.29±0.01	0.21±0.01	0.23±0.02
	C. frutescens	0.33±0.01	0.36±0.02	0.33±0.02	0.34±0.01
40% (w/v)	*E. coracana*	0.37±0.01	0.29±0.01	0.23±0.01	0.26±0.01
	P .vulgaris	12.13±0.37	12.97±0.12	12.00±0.20	13.07±0.18
	R. indica	0.24±0.02	0.20±0.01	0.22±0.01	0.21±0.01
	C. frutescens	0.32±0.02	0.32±0.01	0.30±0.01	0.31±0.02
60% (w/v)	*E. coracana*	0.32±0.02	0.26±0.02	0.21±0.01	0.19±0.02
	P .vulgaris	11.20±0.31	11.60±0.31	10.10±0.15	10.47±0.41
	R. indica	0.22±0.04	0.25±0.01	0.18±0.00	0.20±0.00
	C. frutescens	0.29±0.01	0.29±0.02	0.25±0.01	0.26±0.01

Key

T. p Rw *Tagetes patula* root water extract T. m Rw *Tagetes minuta* root water extract

T.p Lw *Tagetes patula* leaf water extract T. mLw *Tagetes minuta* leaf water extract

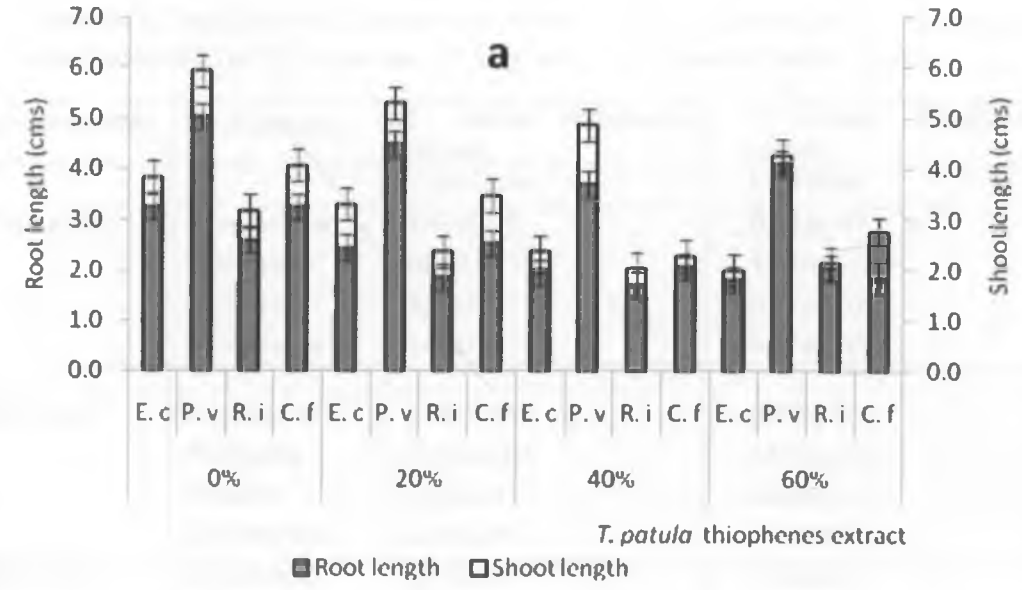
4.7 The influence of thiophene extracts of *T. minuta* and *T. patula* on seedling growth.

Seedling growth response to thiophene extract from the roots of *T. minuta* and *T. patula* was observed to be variable among the test plant seedlings as shown in figures 4.2.4 a & b and table 4.3. Generally, thiophene extracts of *T. patula* and *T. minuta* were more suppressive to the roots of all test plant species followed by the shoot length and least inhibitory to the Leaf area. At 20% thiophene from *T. minuta* the RLs for

E.coracana, P. vulgaris, C. frutescens and *R. indica* was 2.5, 4.6, 2.7 and 2.1 cm respectively, while the SL values of 3.0, 5.5 3.8 and 2.7 cm were recorded and 0.4, 12.2, 0.3 and 0.2 cm2 for LAs respectively. The recorded values of 2.4, 4.5, 2.5 and 1.9 cms for RLs were observed the seedlings of *E.coracana, P. vulgaris, C. frutescens* and *R. indica* treated with 20% thiophene extract from the *T. patula*, similarly the SLs of 3.3, 5.3, 3.5 and 2.4 cms with LAs of 0.4, 11.9, 0.3 and 0.2 cm^2 respctively.

Similarly 40% thiophene extract of *T. minuta* was suppressive resulting in 2.0, 3.9, 2.3 and 1.8 cm for the RL values, while the SL values of 2.5, 5.0, 3.0 and 2.3 cm were recorded and 0.4, 11.3, 0.4 and 0.3 cm^2 values were recorded LAs respectively. At a concentration of 40% *T. patula* thiophenes, RL values of 1.9, 3.7, 2.1 and 1.7 cm for *E.coracana, P. vulgaris, C. frutescens* and *R. indica* with the SL values of 2.4, 4.9, 2.3 and 2.0 cms and LA values of 0.2, 10.8, 0.3 and 0.2 cm^2 were recorded respectively.

Seedlings of *E.coracana, P. vulgaris, C. frutescens* and *R. indica* treated with 60% thiophene extract from *T. minuta* recorded the values of 2.1, 4.1, 1.8 and 1.5 cm for the RL, while values of 1.9, 4.7, 2.7 and 2.3 cm were recorded for the SL and the LAs of 0.2, 10.5, 0.3 and 0.2 cm^2 respectively. On the other hand, the RL values of 1.8, 4.1, 2.8 and 2.0 cm for the seedlings of *E.coracana, P. vulgaris, C. frutescens* and *R. indica* were recorded from the seedlings treated with 60% thiophene extract from *T. patula*. On the same note, the SL values of 2.0, 4.3, 1.8 and 2.1cm and the LA values of 0.2, 10.3, 0.5 and 0.2 cm^2 were recorded for the *E.coracana, P. vulgaris, C. frutescens* and *R. indica* treated with the same extract.



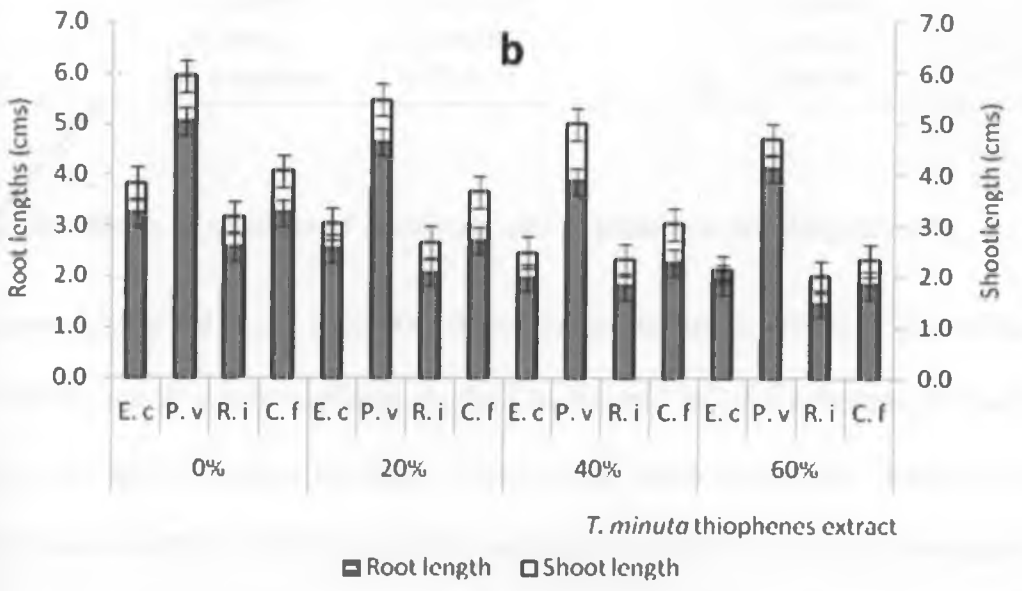


Figure 4.2.4 The effects of thiophenes extract from (a) *T. patula* and (b) *T. minuta* on the root and shoot lengths of *E. coracana, P. vulgaris, C. frutescens* and *R. indica* seedling. Values represent mean±SE, $p<0.05$.

The increase in thiophenes concentration caused a significant reduction in RL and SL as shown by the analysis of variance at $p<0.05$. The response of the leaf area to thiophene extract was negligible and thus was non significantly affected as shown in tables 5a, c and e in the appendix 1.

Table 4.3 The effects of thiophenes extract from *T. minuta* and *T. patula* on the leaf area (mean±SE) of *E. coracana, P. vulgaris, C. frutescens* and *R. indica*

Concentration	Test species	*T. patula* thiophenes extract	*T. minuta* thiophenes extract
		Leaf area	Leaf area
0% (control)	E. coracana	0.42±0.02	0.44±0.01
	P. vulgaris	14.40±0.12	15.40±0.02
	R .indica	0.36±0.01	0.35±0.02
	C. frutescens	0.46±0.01	0.47±0.03
20% (w/v)	E.coracana	0.36±0.01	0.42±0.01
	P.vulgaris	13.53±0.07	13.67±0.35
	R.indica	0.20±0.01	0.22±0.01
	C.rutescens	0.33±0.01	0.34±0.01
40% (w/v)	E.coracana	0.31±0.01	0.36±0.01
	P.vulgaris	12.53±0.41	12.87±0.13
	R.indica	0.20±0.02	0.24±0.02
	C.rutescens	0.23±0.01	0.23±0.01
60% (w/v)	E.coracana	0.24±0.02	0.30±0.01
	P.vulgaris	12.67±0.18	12.80±0.23
	R.indica	0.19±0.01	0.17±0.01
	C.rutescens	0.47±0.01	0.32±0.08

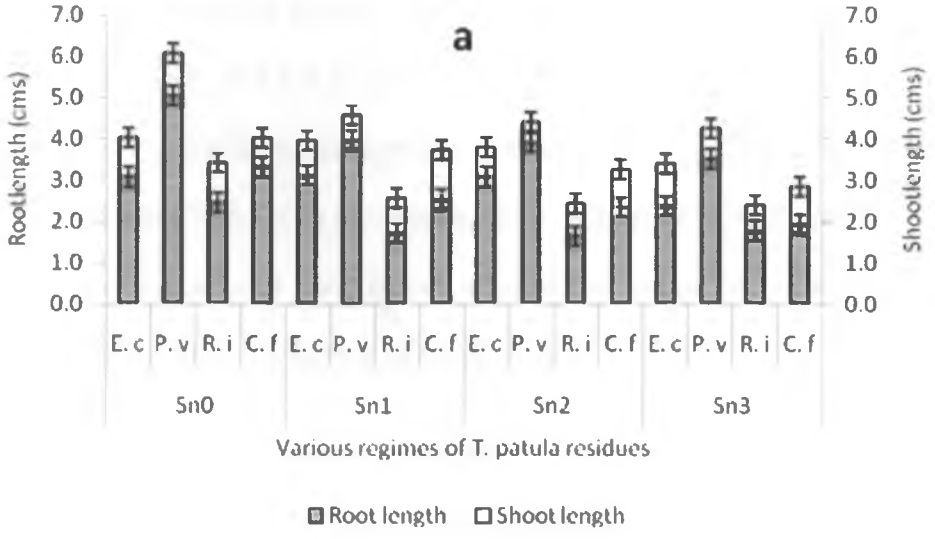
4.8 The effects of residues of *T. minuta* and *T. patula* on seedling growth.

Figures 4.2.5 a and b and Table 4.4, shows the results on the effects of the residues from *T. minuta* and *T. patula* seedlings on the LA, RL and SL of *E.coracana, P. vulgaris, C. frutescens* and *R. indica* seedlings. The results indicated that the residues caused a significant suppressive effect on the RL and the SL of all the test species compared to the control plants. The LA values of 0.35, 14.8, 0.54 and 0.32 cm^2, while the RL values of 3.1, 5.1, 3.3 and 2.5 cm and the shoot lengths of 4.0, 6.1, 4.1 and 3.4 cm were recorded for *E.coracana, P. vulgaris, C. frutescens* and *R. indica* seedlings in the control treatment.

All the three residue regimes of *T. minuta* and *T. patula* exhibited the suppression effect on the growth of roots and shoots with the highest suppression effect noted in Sn2

and Sn3. RL values of 3.3, 4.9, 2.3 and 2.1 cm with SL values of 4.1, 5.8, 3.7 and 2.4 cms and LA values of 0.3, 13.3, 0.3 and 0.2 cm^2 were recorded for *E.coracana, P. vulgaris, C. frutescens* and *R. indica* seedings that were established in Sn1 of *T. minuta.* On the other hand,RL values of 3.1, 4.7, 2.3 and 1.7 cm with SL values of 4.0, 5.8, 3.7 and 2.6 cm and LAs of 0.4, 13.2, 0.3 and 0.2 cm^2 were recorded for *E.coracana, P. vulgaris, C. frutescens* and *R. indica* respectively.

The growth parameters of the seedlings that were subjected to residues of regime Sn2 were highly reduced as shown in figures 4.2.6. RL values of 3.1, 4.5, 2.3 and 1.9 cm with SL vlues of 3.8, 5.7, 3.3 and 2.6 cm and LA values of 0.3, 12.9, 0.3 and 0.2 cm^2 values were recorded in seedlings of *E.coracana, P. vulgaris, C. frutescens* and *R. indica* that were established in Sn2 residues of *T. minuta.* Seedlings of *.coracana, P. vulgaris, C. frutescens* and *R. indica* registered RL values of 3.1, 4.9, 2.3 and 1.6 cm with SL values of 3.8, 5.9, 3.3 and 2.4 cm and LA values of 0.3, 12.5, 0.3 and 0.2 cm^2 in Sn2 residues of *T. patula* .



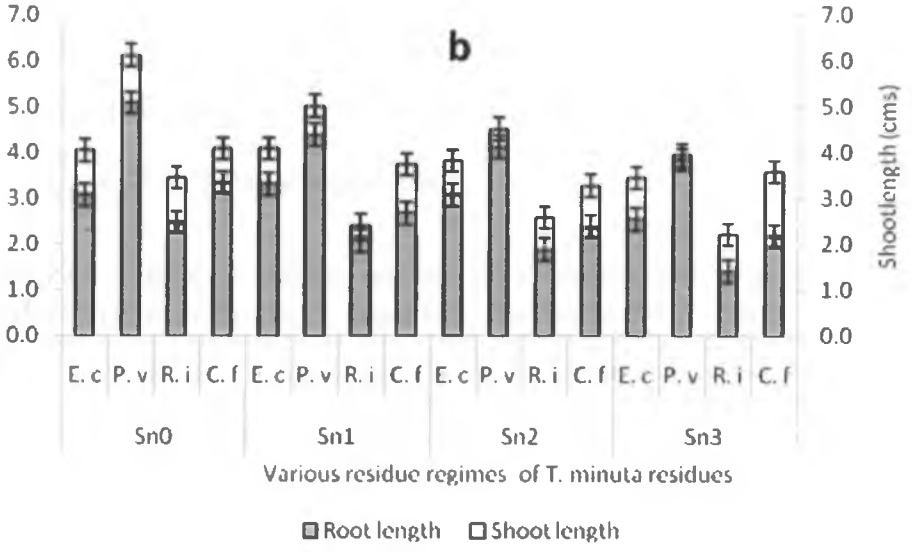


Figure 4.2.5 The effects of residue regimes of (a) *T. patula* and (b) *T. minuta* on the root and shoot length of *E. coracana, P. vulgaris, C. frutescens* and *R. indica* seedlings. Values represent the mean±SE, $p<0.05$.

The RL, SL and LA were severely affected by the residues of Sn3 in both *T. minuta* and *T. patula.* Seedlings of *E.coracana, P. vulgaris, C. frutescens* and *R. indica* that were established in residues of Sn3 of *T. minuta*, registered RL values of 2.5, 3.9, 2.6 and 1.4 cm, with SL values of 3.4, 4.9, 3.6 and 2.2 cms and LA values of 0.2, 11.1, 0.4 and 0.2 cm^2 respectively. Similarly, the seedling of *E.coracana, P. vulgaris, C. frutescens* and *R.*

established in residues of Sn3 of *T. minuta*, registered RL values of 2.5, 3.9, 2.6 and 1.4 cm, with SL values of 3.4, 4.9, 3.6 and 2.2 cms and LA values of 0.2, 11.1, 0.4 and 0.2 cm^2 respectively. Similarly, the seedling of *E.coracana, P. vulgaris, C. frutescens* and *R. indica* established in the soils incorporated with residues of *T. patula* of Sn3, recorded the RL values of 2.4, 4.7, 2.3 and 1.8 cms , while the SL values of 3.4, 5.8, 2.8 and 2.4 cms with LAs of 0.2, 11.8, 0.3 and 0.2 cm^2 respectively.

It was also observed that the magnitude reduction in the SL and d RL for each test plant was dependent on the level of the residue regime. At Sn3 the SL and RL were significantly reduced as compared to those seedlings in the control experiment. This was confirmed by the post hoc test of comparison using the LSD test, which indicated that at higher residual regime (Sn3), the SL and RL values were significantly reduced at $p<0.05$ as shown in tables 5 d & e in appendix 1.

Table 4.4 Effects of residue regimes of *T. minuta* and *T. patula* on the leaf area (mean±SE) of *E. coracana, P. vulgaris, C. frutescens* and *R. indica*

Residue regime	Test plant species	Leaf area (cm^2) response to residues of *T. patula*	Leaf area (cm^2) response to *T. minuta* residue
Sn0 (control)	*E. coracana*	0.35±0.02	0.34±0.01
	P.vulgaris	14.80±0.23	14.20±0.20
	R. indica	0.32±0.02	0.30±0.01
	C. frutescens	0.54±0.01	0.55±0.02
Sn1	*E. coracana*	0.36±0.01	0.34±0.01
	P. vulgaris	13.23±0.26	13.27±0.19
	R. indica	0.23±0.01	0.21±0.01
	C. frutescens	0.32±0.01	0.32±0.01
Sn2	*E. coracana*	0.31±0.01	0.31±0.01
	P. vulgaris	12.47±0.18	12.87±0.18
	R. indica	0.21±0.01	0.23±0.01
	C. frutescens	0.33±0.01	0.33±0.01
Sn3	*E. coracana*	0.22±0.00	0.23±0.01
	P. vulgaris	11.80±0.20	11.13±0.18
	R. indica	0.22±0.01	0.21±0.01
	C. frutescens	0.28±0.03	0.35±0.02

Key Sn= residue regime; 1, 2, 3 = number of times the seedlings were grown.

CHAPTER FIVE

5.0 DISCUSSION

5.1 Response of test plants to the *T. minuta* and *T. patula* extracts and residues.

The herbicidal potential of plants is often defined by their ability to displace other plants from their natural habitats, while they establish stable populations. *Tagetes minuta* and *Tagetes patula* have been known to possess some of these characteristics, which are often attributed to the presence of allelochemicals which have the herbicidal effect (Daizy *et al.,* 2007). Imbibition of these allelochemicals from the soil antagonizes various physiological and biochemical mechanisms which lead to impaired embryo development and seedling growth.

Consequently, a number of susceptible plants are inhibited and their populations are greatly reduced by the allelochemicals which are released into the soil. Alsaadawi *et al.,* (1990) showed that allelopathic plants can determine the plant structure in a natural habitat through the release of allelochemicals. In this study, the herbicidal potential of *T. minuta* and *T. patula* extracts on the germination of *E. coracana, P. vulgaris, C. frutescens* and *R. indica* seeds was assessed by seed germination percentage and germination delay.

The observed reduction in the mean germination percentages of test plants did reveal a relationship between the degree of its reduction and the proportion of its extracts. This relationship is shown by the high inhibition at a maximum concentration and could be explained by the fact that high concentrations of the extracts contain more active ingredients per definite volume of the solvent compared to low concentrations of the

extracts. Therefore, the low germination percentages of the test plants are indicative of the increased sensivity at maximum concentration. This observation is supported by the work of Kil *et al.,* 2002 who reported that the inhibition effect of *T. minuta* aqueous extracts on *Lotus corniculatus var. japonicas* and *Lactuca sativa* seeds increased with increase in concentration.

5.2 The germination and earlier seedling growth responses to water extracts of *T. patula* and *T. minuta*.

It's apparent that water extracts from the roots and leaves of *T. patula* and *T. minuta* revealed the increasing herbicidal effect on test plants with respect to increasing concencentration. Subsequently, the leaves exhibited high germination inhibition effect compared to the roots and this can be explained by the fact that leaves of allelopathic plants contain more allelochemicals compared to the roots (Ashraf *et al.,* 2008). This is because the leaves are the fundamental sites for the major biosynthetic pathways which lead to the formation of primary, secondary and intermediate compounds compared to the roots which are the sites of entry of raw materials into the plant systems. This is justified by the low germination percentages on the seeds treated by the water extracts from the leaves compared to roots. Similar observations have been made by Raffique *et al.,* 2003; Ejaz *et al.*, 2003; Siddiqui *et al.,* 2009 who revealed that the leaf water extracts of different agroforestry trees were inhibitory to seed germination of various plants.

The responses would reflect the possible application of the water extracts from the leaves of both *T. minuta* and *T. patula* as a pre-emergence herbicide to weed germination as compared to water extracts from the roots. This suggestion is similarly supported by the work of Batish *et al.,* 2007, who demonstrated the potential utilization of *T. minuta* leaves as a natural herbicide in the management of weeds in rice fields.

The varied sensitivity of the test plants to the water extracts from both leaves and roots of *T. minuta* and *T. patula* was evidenced by the different germination percentages obtained for each test plant. These diversified responses may be attributed to the seed size, permeability of the testa coat to extracts and the surface area to volume ratio presented by the test plant seeds (Perez, 1990). Similar findings have been reported by Yarnia *et al.*, 2009 who showed that seeds of *Amaranthus retroflexus* were highly inhibited by the *Hordeum* extract compared to the seeds of *Portulaca oleracea, Digittaria ischiamum L* and *Chenopodium album* since they are small in size and contains very little amount of carbohydrates.

The germination delay varied a mong the test plants may be attributed to the differences of their sensitivity to the extracts. These findings are in agreement with the work of Phiri and Mbewe, 2010 who revealed that extracts of *Moringa oleifera* leaves prolonged the germination of beans (*Arachis hypogea, Phaseolus vulgaris* and *Vigna ungulata* (L) Walp by 100%.

The delay in germination signify the herbicidal potential of *T. minuta* and *T. patula* can be utilized to suppress susceptible weeds before planting of the crop plants in this case *P. vulgaris*. In regard to this, Benyas *et al.*, 2010 has revealed that the aqueous extracts of *Xanthium strumarium* L. not only decreased the germination rate of *Lens culinaris*, but also lead to delayed emergence and poor establishment of lentil seedlings.

The herbicidal potential of *T. minuta* and *T. patula* was further noted on seedlings of *R. indica, E. coracana, C. frutescens* and *P. vulgaris* as evidenced by reduction in root length and shoot length. Roots of all test plants exhibited high sensitity to the water extracts at all concentrations as compared to the shoot lengths and this was noted by the greater magnitude of length reduction in roots. These results agree with the findings of

Turk and Tawaha, 2002; Ashrafi *et al.*, 2008 who demonstrated the severe reduction effect on root lengths of lentil compared to the shoot lengths by water extracts from barley plant. The herbicidal potential on seedling growth increased with increase in extract concentration. This can be explained by the high amount of the growth inhibitors contained in extracts at maximum concentration. This has also been reported by Rahimi *et al.*, 2006; Benyas *et al.*, 2010 who revealed that reduction in root length of wild barley, pigweed, mustard, Lambesquarter and lentil by *Plantago psyllium* and *Xanthium strumarium* was propotional to the extract concentration.

The herbicidal potential of *T. minuta* and *T. patula* was non different as evidenced by the similar ranges of MGPs reduction of the test plants. This could be explained by the content of allelochemicals contained by the water extracts from these two plants. Furthermore, the action of these allelochemicals from *T. minuta* and *T. patula* may have similar site of action on the target plant. Therefore, the physiological processes suppressed by the extracts of these two plants retard the germination of the test plant almost to a similar level. In addition, these plants belong to the same genus, thus indicating that the chemicals synthesized by these plants may be of the same class and even of the same quantity and concentration.

5.3 The influence of methanol extracts of *T. minuta* and *T. patula* on the germination and growth of the test plants.

Plants metabolise a wide spetra of secondary metabolites whose solubilities vary greatly depending on their polarity. Some of the plant chemicals are soluble in water, while others in organic solvents including the methanol and others have been isolated through a mixture of solvents. Methanol was chosen for the extraction of organic solvent because of

its volatility, thus ensuring that the solvent is totally removed from the extract and their physiological activities are not altered.

Therefore, in this study the herbicidal potential of *T. minuta* and *T. patula* was further exhibited by the methanol extract from the leaves and roots as evidenced by reduced germination percentage of *E. coracana, P. vulgaris, C. frutescens* and *R. indica* seeds. It is apparently clear that the degree of seed germination inhibition increased with increase in concentration, though the magnitude of inhibition varied among the test species. Comparatively, it is worthy noting that the methanol extracts had a higher germination inhibition effect on seed germination compared to water extracts, however the reduction range is very close. This implies that both water and methanol solvents have the potential of extracting active ingredients from *T. minuta* and *T. patula* with the herbicidal properties. This observation is supported by the work of Salim and Noguchi, 2010 who demonstrated that methanol extract from 14 Bangladesh rice cultivars had a strong inhibitory effect on the germination of *Lepidium sativum, Digitaria sanguinalish* and *Phleum pretense*.

High inhibition was observed in test plant seeds treated with the methanol extracts from the leaves at all concentrations. It was also evident that the methanol extracts from the leaves of *T. patula* and *T. minuta* had the highest inhibiton on the germination of seeds compared to the root methanol extracts. This can be supported by the observed low MGP in seeds of the test plants treated with the leaf methanol extracts as compared to those treated with the root. Therefore, this is an indication that the leaves contain high content of active ingredients that are inhibitory to germination and that they are not uniformly distributed in all plant organs. Similar evidence is reported by Turk and Tahawa, 2002,

who demonstrated pronounced inhibitory effect on seed germination by leaf extract treatments.

These results are related to the work done by Tsao *et al.,* 2002, which revealed strong germination inhibitory effect of methanol extracts from the fresh leaves of *Ailanthus altissima* L. on alfalfa (*Medicago sativa*) seeds. In this context, the results have shown that both water and methanol extracts from *T. minuta* and *T. patula* contain active ingredients that can be utilized as bio-herbicides in regulating the germination of seeds of both economic and weed plants. However, the effectivity of the extract on germination inhibition is shown to be dependent on its concentration. The challenge that arises in field applications of such results involves the determination of the appropriate concentration that would be effective for the different plant species.

The observed germination delay for each test species can be attributed to the fact that the imbibed allelochemicals limited the embryonic cell division, cell elongation and deactivation of hydrolytic enzymes which initiate the protrution of the radicle (Rice, 1984; Rizvi and Rizvi, 1992). This is further evidenced by the prolonged period of germination time taken for each species to germinate compared to the control. This observation coincides with the findings of Angiras *et al.,* 1988; Ghaffar *et al.,* 2000 who reported that sunflower and barley methanol extracts significantly delayed the germination of wheat and other weeds respectively.

The herbicidal potential of the methanol extracts was further demonstrated by the reduction of the root and shoot length of the test plant seedlings. This trend of reduction expresses the potential application of the methanol extract as a post emergence herbicide for the management of weeds. However, roots were more sensitive as compared to the shoots and this can be explained by the fact that, root absorb the active ingredients before

they are modified by the plants'detoxification mechanisms. These results are consistent with the findings of Sadeghi *et al.*, 2010, who demonstrated the suppression of the shoot and root of wheat (*Triticum aestivum*) by the sunflower (*Helianthus annus*) and barley (*Hordeum vulgare* L.).

Furthermore, the reduction was observed to be proportional to the concentration of the methanol extract, with minimum root length recorded at the maximum concencentration. This is supported by the work of Katu-Noguchi, 2002; Chung *et al.,* 2002; Sharma *et al.,* 2009 who demonstrated herbicidal potential of the methanol extracts on the growth of various plants, for example, methanol extract from bryophytes (*Targonia hypophylla, Marchantia polymorpha, plagiochasma appendiculatum, Brachythecium buchananii, Leucodon secundus, Timmiella anomala, Rhoobryum roseum* and *Plagiomnium integram)* on *Bidens biternata* was shown to be dependent on the concentration.

5.4 The germination and growth response of selected plant species to thiophenes extracts of *T. patula* and *T. minuta*

Thiophenes are among the diversity of allelochemicals which occur in *T. patula* and *T. minuta*, whose herbicidal activity has not been fully explored. Greater amounts of this compound accumulate in the roots of these plants compared to the leaves and other parts of the plant (Ketel, 1987). For substantial amount of extract, thiophene is extracted from the roots through solvent mixtures and then bioassayed on the test species. Bioactivity of thiophenes is enhanced by light energy especially ultraviolet light.

Therefore, in this study the herbicidal potential of *T. patula* and *T. minuta* was further accessed through thiophene extracts on the germination and growth of *R. indica, E. coracana, P. vulgaris* and *C. frutescens*. The reduction in the MGP of the test plants was

a clear indicator of the hercidal potential of thiophenes on seed germination; this was enhanced at high concentrations. The sensitivity to thiophenes tended to vary among the test plants as evidenced by the low germination for *R. indica* and relatively high germination percentages for *E. coracana, P. vulgaris* and *C. frutescens* at all levels of extract concentration. This may suggest that though thiophenes may have specific sites to inhibit, the differences may arise from the amount imbibed and the presence of carbohydrates in the test plant seeds.

The herbicidal potential of thiophenes depended on concentration, and thus the observed lower germination percentages at maximum concentrations were a manifestation of this phenomenon, although the MGPs of the test plants were statistically non significant. The MGP and MGT values were relatively high for the seeds treated with thiophene extracts compared to those seeds that were subjected to the water and the methanol extracts. This could be attributed to the amount of the active ingredients in thiophenes that were imbibed into the germinating seeds. Therefore, more work is recommended to elucidate the mechanisms in which thiophenes work to inhibit seed germination, cell division and elongation, membrane permeability and enzymatic activities.

Campbell *et al.,* (1982), proposed that the germination response of test plants to thiophenes may involve a complex of physiological processes in which the permeability of the membrane is reduced. Therefore, the ability of the seed to imbibe water and minerals is impaired, thus reducing the chances of germination. This is further supported by the response of MGP of test plants which did show proportional decline as the concentration of thiophenes increased. The work of Nikolaus and Leovigildo, 1985

supports this observation by revealing that thiophenes from the roots of *Ambrosia artemisiifolia* regulated the germination of many plant species.

The herbicidal potential of *T. minuta* and *T. patula* was further revealed by the germination delay. Seeds of test plants treated with thiophene extracts from *T. minuta* and *T. patula* showed a varied response. The variation in time of germination for each test species is an indication that thiophenes can be applied to delay the germination of weeds before planting. These mechanisms tend to vary among the test plant as shown by *R. indica* that took long time to germinate compared to *P. vulgaris* and *E. coracana.* In regard to this, the herbicidal potential of thiophenes increased with the increase in concentration, as indicated by low MGTs of the test plants at the maximum concentrations. Therefore, concentration seems to be a critical parameter in deriving thiophenes from *T. minuta* and *T. patula* for weed management

Thiophenes seem to possess both pre and post emergence herbicidal characteristics. As was observed for the pre emergence effect of thiophenes, similarly, there was herbicidal effect on seedlings of the test plants. This was indicated by the reduction in root and shoot lengths of the test plants, although the strength of thiophenes was weak compared to water and the methanol extracts. This weak herbicidal signal of thiophene may be due to the lack of synergic effect as in the case of water and methanol extract. Similarly the results indicated that the roots continued to exhibit high sensitivity to thiophenes compared to shoot length for each test plant. This high sensitivity may be due to the fact that roots are the sites of inhibitor entry into the plant system in undulated state. In a similar case, the observed increased response to increase in concentration helps to confirm the idea that the roots were more sensitive to the faster accumulation of thiophenes in the root cells as compared to the shoot and in the leaves.This is supported

by the work of Kong *et al.,* 2007, who demonstrated the allelopathic interference of *Ambrosia trifida* against wheat (*Triticum aestivum*).

5.5 The effect of residues of *T. minuta* and *T. patula* on the germination and growth of selected plant species.

Plant residues on the soil surface or incorporated in the soil are sometimes harmful to plant germination and earlier seedling growth through release of phytotoxic compounds during decomposition (Chou and Lin, 1976). Residues from the plants releases greater amount of allelochemicals to the soil compared to one organ. This can be proved by the fact that various plant metabolites are stored in different plant organs, including roots, leaves, bark, leaves aand flowering parts (Ketel, 1987). During decomposition, each organ releases its allelochemicals into the soil, therefore the expected high reduction effect on the germination and growth of the test plants.

Therefore, in this study, the intergral herbicidal potential of *T. minuta* and *T. patula* was highly pronounced when the whole seedlings were incorporated into the soil. Unlike the water and methanol extracts from the roots and leaves, residues of *T. minuta* and *T. patula* reduced greatly inhibited seed germination as evidenced by the lowly recorded MGPs of *E. coracana, P. vulgaris, C. frutscens* and *R. indica*. This observation could be explained by the greater contribution of allelochemicals from all plant parts of *T. minuta* and *T. patula* residues. The higher inhibitory effect observed on seeds sown at regime three, indicated an increase in herbicidal potential with the quantity of *T. patula* and *T. minuta* incorporated in the soil. These results are in accordance with the studies of Bhagarith and David, 2009 who demonstrated that the reduction on the emergence of junglerice *(Echinochlora colona)* seeds increased with increasing quantities of residues in the soil.

Further, the response of test plants varied greatly, as evidenced by the fact that *R. indica* and *C. frutescens* exhibited high inhibition to the residues compared to *P. vulgaris* and *E. coracana*. As explained earlier, this can be attributed to the rate at which allelochemicals are imbibed by the seed, permeability of the testa, amount of carbohydrates and the general sensitivity of embryo cells to allelochemicals. This observation agree with the findings of Prezepiorkowski and Stanley, 1994, who revealed high germination inhibitory effect of Rye (*Secale cereale*) residues on barnyardgrass (*Echnochloa crus-galli*), horseweed (*Conyza canadensis*) and willowherb (*Epilobium ciliatum*)

The pre emergence herbicidal potential of *T. minuta* and *T. patula* was demonstrated by prolonged germination period. The delay was an indication that the released allelochemicals from the decomposing materials were imbibed by the test plant seeds, leading to the reduced germination in treated alteration seed. Similar suggestion has been reported by Onwogbuta, 2001 who showed that residues of *Chromolaena odorata* L. did reduce the germination of tomatoes (*Lycopersicum esculentum*).

Besides the germination inhibition and delay, residues also reduced the elongation of the root and shoot lengths of *E. coraca, P. vulgaris, C. frutescens* and *R. indica* seedlings. The sensitivity to the residues was similar as was with other treatments, thus roots were observed to be highly sensitive compared to the shoot length and the leaf area that indicated no response to residue regimes. This is evidenced by the short root lengths recorded compared to the shoot lengths, though the response was independent for each test plant.

5.6 Conclusion

The biological activities of the extracts on the test plants revealed the herbicidal potential of *T. minuta* and *T. patula* plants. Seed germination inhibition and germination delay induced by these extracts showed that aromatic plants can be used for the management of weeds. The varied sensitivity of the test plants to the extracts indicates the aspect of selectivity of these plants to tagetes allelochemicals. *R. indica* was the most sensitive to all extracts at all levels of concentrations, while *C. frutescens, E. coracana* and *P. vulgaris* registered aprogressive decline in mean germination percentage as the concentration of the extracts increased. Similarly, the germination time for each test plant was shown to be dependent on the concentration of the extract. Germination inhibition and seedling growth suppression was great in the residue, water and methanol treatment compared to thiophene. Roots of the test plants were shown to be the most affected by the extracts, followed by the shoot length, though the leaf area was non significantly affected. The results obtained in this study indicate that extracts from *Tagetes minuta* and *T. patula* can be used as the natural herbicides to manage the weeds in farming land if grown in large scale.

Recommendation

1. *Tagetes minuta* and *T. patula* have been shown to have the herbicidal property and thus they can be used to manage weeds in agricultural farms.
2. The varied sensitivity exhibited by the test plants to the extracts, showed that the bioactive compounds in *T. minuta* and *T. patula* can be used to selectively eliminate various weeds growing between the economic crops.
3. The germination suppression was shown to be depended on the concentration of the extract; therefore establishment of the effective dose concentration is recommended for proper weed elimimnation.
4. Extracts from the leaves and residues were shown to have the highest suppression effect on germination and seedling growth, these indicates that they contain higher amount of active compounds, therefore they are recommended for use as the best sources for the extraction of the bioactive compounds.

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Appendix 1

Table 1a; ANOVA table indicating the effects of the extracts from the roots of *T. minuta* and *T.*

			Sum of Squares	df	Mean Square	F	Sig.
MGP * extract	Between Groups	(Combined)	839.791	4	209.948	8.843	.001
	Within Groups		2659.122	112	23.742		
	Total		3498.913	116			
MGT * extract	Between Groups	(Combined)	3.825	4	.956	8.164	.001
	Within Groups		13.118	112	.117		
	Total		16.943	116			

patula on the MGT of *E. coracana*

Table 1b; The comparison of each extract and residue from *T. minuta* and *T. patula* between on the mean germination percentage of *E. corcana*
Dependent Variable: MGP

(I) extract	(J) extract	Mean Difference (I-J)	Std. Error	Sig.
Control	Water E	7.34568(*)	2.05128	.001
	methanol	7.22222(*)	2.05128	.001
	thiophene	8.64198(*)	2.24707	.000
	residue	10.49383(*)	2.24707	.000
water	Control	-7.34568(*)	2.05128	.001
	methanol	-.12346	1.29735	.924
	thiophene	1.29630	1.58892	.416
	residue	3.14815	1.58892	.050
methanol	Control	-7.22222(*)	2.05128	.001
	Water E	.12346	1.29735	.924
	thiophene	1.41975	1.58892	.373
	residue	3.27160(*)	1.58892	.042
thiophene	Control	-8.64198(*)	2.24707	.000
	Water E	-1.29630	1.58892	.416
	methanol	-1.41975	1.58892	.373
	residue	1.85185	1.83472	.315
residue	Control	-10.49383(*)	2.24707	.000
	Water E	-3.14815	1.58892	.050
	methanol	-3.27160(*)	1.58892	.042
	thiophene	-1.85185	1.83472	.315

* The mean difference is significant at the .05 level.

Table 1c; The contribution of each extract concentration on the on the MGP *of E. coracana.* The * indicate the significant difference between the means at P<0.05

(I) concentration	(J) concentration	Mean Difference (I-J)	Std. Error	Sig.
0%	20%	2.14815	1.37644	.121
	40%	7.40741(*)	1.37644	.001
	60%	13.11111(*)	1.37644	.001
	Gen1	6.29630(*)	1.90878	.001
	Gen2	11.48148(*)	1.90878	.001
	Gen3	13.70370(*)	1.90878	.000
20%	0%	-2.14815	1.37644	.121
	40%	5.25926(*)	.93511	.001
	60%	10.96296(*)	.93511	.001
	Gen1	4.14815(*)	1.61965	.012
	Gen2	9.33333(*)	1.61965	.001
	Gen3	11.55556(*)	1.61965	.001
40%	0%	-7.40741(*)	1.37644	.001
	20%	-5.25926(*)	.93511	.001
	60%	5.70370(*)	.93511	.001
	Gen1	-1.11111	1.61965	.494
	Gen2	4.07407(*)	1.61965	.013
	Gen3	6.29630(*)	1.61965	.001
60%	0%	-13.11111(*)	1.37644	.001
	20%	-10.96296(*)	.93511	.001
	40%	-5.70370(*)	.93511	.001
	Gen1	-6.81481(*)	1.61965	.001
	Gen2	-1.62963	1.61965	.317
	Gen3	.59259	1.61965	.715
Gen1	0%	-6.29630(*)	1.90878	.001
	20%	-4.14815(*)	1.61965	.012
	40%	1.11111	1.61965	.494
	60%	6.81481(*)	1.61965	.001
	Gen2	5.18519(*)	2.09096	.015
	Gen3	7.40741(*)	2.09096	.001
Gen2	0%	-11.48148(*)	1.90878	.001
	20%	-9.33333(*)	1.61965	.001
	40%	-4.07407(*)	1.61965	.013
	60%	1.62963	1.61965	.317
	Gen1	-5.18519(*)	2.09096	.015
	Gen3	2.22222	2.09096	.290
Gen3	0%	-13.70370(*)	1.90878	.001
	20%	-11.55556(*)	1.61965	.001
	40%	-6.29630(*)	1.61965	.001
	60%	-.59259	1.61965	.715
	Gen1	-7.40741(*)	2.09096	.001
	Gen2	-2.22222	2.09096	.290

Table 1d; The multiple comparison table showing the effect of each extracts on the MGT of *E. coracana.* (* The mean difference is significant at the .05 level.)

(I) extract	(J) extract	Mean Difference (I-J)	Std. Error	Sig.
control	Water E	-.26320(*)	.11817	.028
	methanol	-.53871(*)	.11817	.001
	thiophene	-.55374(*)	.12945	.001
	residue	-.56021(*)	.12945	.001
waterE	control	.26320(*)	.11817	.028
	methanol	-.27551(*)	.07474	.001
	thiophene	-.29054(*)	.09153	.002
	residue	-.29701(*)	.09153	.002
methanol	control	.53871(*)	.11817	.001
	Water E	.27551(*)	.07474	.001
	thiophene	-.01503	.09153	.870
	residue	-.02151	.09153	.815
thiophene	control	.55374(*)	.12945	.001
	Water E	.29054(*)	.09153	.002
	methanol	.01503	.09153	.870
	residue	-.00647	.10570	.951
residue	control	.56021(*)	.12945	.001
	Water E	.29701(*)	.09153	.002
	methanol	.02151	.09153	.815
	thiophene	.00647	.10570	.951

* The mean difference is significant at the .05 level.

Table 1e; Shows the LSD among the various extract concentration on the MGT of *E. coracana*

(I) concentration	(J) concentration	Mean Difference (I-J)	Std. Error	Sig.
0%	20%	-.07069	.08468	.406
	40%	-.47406(*)	.08468	.001
	60%	-.74977(*)	.08468	.001
	Gen1	-.49039(*)	.11743	.001
	Gen2	-.39927(*)	.11743	.001
	Gen3	-.79097(*)	.11743	.000
20%	0%	.07069	.08468	.406
	40%	-.40337(*)	.05753	.001
	60%	-.67907(*)	.05753	.001
	Gen1	-.41970(*)	.09964	.001
	Gen2	-.32858(*)	.09964	.001
	Gen3	-.72028(*)	.09964	.001
40%	0%	.47406(*)	.08468	.001
	20%	.40337(*)	.05753	.001
	60%	-.27571(*)	.05753	.001
	Gen1	-.01633	.09964	.870
	Gen2	.07479	.09964	.454
	Gen3	-.31691(*)	.09964	.002
60%	0%	.74977(*)	.08468	.001
	20%	.67907(*)	.05753	.001
	40%	.27571(*)	.05753	.001
	Gen1	.25938(*)	.09964	.011
	Gen2	.35050(*)	.09964	.001
	Gen3	-.04120	.09964	.680
Gen1	0%	.49039(*)	.11743	.001
	20%	.41970(*)	.09964	.001
	40%	.01633	.09964	.870
	60%	-.25938(*)	.09964	.011
	Gen2	.09112	.12863	.480
	Gen3	-.30058(*)	.12863	.021
Gen2	0%	.39927(*)	.11743	.001
	20%	.32858(*)	.09964	.001
	40%	-.07479	.09964	.454
	60%	-.35050(*)	.09964	.001
	Gen1	-.09112	.12863	.480
	Gen3	-.39170(*)	.12863	.003
Gen3	0%	.79097(*)	.11743	.001
	20%	.72028(*)	.09964	.001
	40%	.31691(*)	.09964	.002
	60%	.04120	.09964	.680
	Gen1	.30058(*)	.12863	.021
	Gen2	.39170(*)	.12863	.003

* The mean difference is significant at the .05 level.

Table 2a Effects of the various extracts on the MGP and MGT of *P. vulgaris*

ANOVA Table

				Sum of Squares	df	Mean Square	F	Sig.
MGP extract	*	Between Groups	(Combined)	839.791	4	209.948	8.843	.001
		Within Groups		2659.122	112	23.742		
		Total		3498.913	116			
MGT extract	*	Between Groups	(Combined)	3.825	4	.956	8.164	.001
		Within Groups		13.118	112	.117		
		Total		16.943	116			

Table 2b Multiple Comparisons of the various extracts on the MGP of *P. vulgaris* (LSD)

(I) extract	(J) extract	Mean Difference (I-J)	Std. Error	Sig.
Control	Water E	6.79012(*)	1.81591	.001
	methanol	8.20988(*)	1.81591	.001
	thiophene	10.49383(*)	1.98923	.001
	residue	10.37037(*)	1.98923	.001
WaterE	Control	-6.79012(*)	1.81591	.001
	methanol	1.41975	1.14848	.219
	thiophene	3.70370(*)	1.40660	.010
	residue	3.58025(*)	1.40660	.012
methanol	Control	-8.20988(*)	1.81591	.001
	Water E	-1.41975	1.14848	.219
	thiophene	2.28395	1.40660	.107
	residue	2.16049	1.40660	.127
thiophene	Control	-10.49383(*)	1.98923	.000
	Water E	-3.70370(*)	1.40660	.010
	methanol	-2.28395	1.40660	.107
	residue	-.12346	1.62420	.940
residue	Control	-10.37037(*)	1.98923	.001
	Water E	-3.58025(*)	1.40660	.012
	methanol	-2.16049	1.40660	.127
	thiophene	.12346	1.62420	.940

* The mean difference is significant at the .05 level.

Table 2c Multiple comparisons of the various extract concentrations on the MGP of *P. vulgaris*

(I) concentration	(J) concentration	Mean Difference (I-J)	Std. Error	Sig.
0%	20%	3.67901(*)	1.40870	.010
	40%	8.19753(*)	1.40870	.001
	60%	12.41975(*)	1.40870	.001
	Gen1	6.41975(*)	1.95351	.001
	Gen2	10.86420(*)	1.95351	.001
	Gen3	13.82716(*)	1.95351	.001
20%	0%	-3.67901(*)	1.40870	.010
	40%	4.51852(*)	.95702	.000
	60%	8.74074(*)	.95702	.000
	Gen1	2.74074	1.65761	.101
	Gen2	7.18519(*)	1.65761	.001
	Gen3	10.14815(*)	1.65761	.001
40%	0%	-8.19753(*)	1.40870	.001
	20%	-4.51852(*)	.95702	.001
	60%	4.22222(*)	.95702	.001
	Gen1	-1.77778	1.65761	.286
	Gen2	2.66667	1.65761	.111
	Gen3	5.62963(*)	1.65761	.001
60%	0%	-12.41975(*)	1.40870	.001
	20%	-8.74074(*)	.95702	.001
	40%	-4.22222(*)	.95702	.001
	Gen1	-6.00000(*)	1.65761	.001
	Gen2	-1.55556	1.65761	.350
	Gen3	1.40741	1.65761	.398
Gen1	0%	-6.41975(*)	1.95351	.001
	20%	-2.74074	1.65761	.101
	40%	1.77778	1.65761	.286
	60%	6.00000(*)	1.65761	.001
	Gen2	4.44444(*)	2.13997	.040
	Gen3	7.40741(*)	2.13997	.001
Gen2	0%	-10.86420(*)	1.95351	.000
	20%	-7.18519(*)	1.65761	.000
	40%	-2.66667	1.65761	.111
	60%	1.55556	1.65761	.350
	Gen1	-4.44444(*)	2.13997	.040
	Gen3	2.96296	2.13997	.169
Gen3	0%	-13.82716(*)	1.95351	.001
	20%	-10.14815(*)	1.65761	.001
	40%	-5.62963(*)	1.65761	.001
	60%	-1.40741	1.65761	.398
	Gen1	-7.40741(*)	2.13997	.001
	Gen2	-2.96296	2.13997	.169

* The mean difference is significant at the .05 level.

Table 2d; multiple comparison table of the various extracts on the MGT of *P. vulgaris*

LSD

(I) extract	(J) extract	Mean Difference (I-J)	Std. Error	Sig.
Control	Water E	-.70541(*)	.12755	.001
	methanol	-.49507(*)	.12755	.001
	thiophene	-.62015(*)	.13972	.001
	residue	-.59778(*)	.13972	.001
Water E	Control	.70541(*)	.12755	.001
	methanol	.21034(*)	.08067	.010
	thiophene	.08526	.09880	.390
	residue	.10763	.09880	.278
methanol	waterC	.49507(*)	.12755	.001
	Water E	-.21034(*)	.08067	.010
	thiophene	-.12509	.09880	.208
	residue	-.10272	.09880	.301
thiophene	Control	.62015(*)	.13972	.001
	Water E	-.08526	.09880	.390
	methanol	.12509	.09880	.208
	residue	.02237	.11408	.845
residue	Control	.59778(*)	.13972	.001
	Water E	-.10763	.09880	.278
	methanol	.10272	.09880	.301
	thiophene	-.02237	.11408	.845

* The mean difference is significant at the .05 level.

Table 2e; Multiple comparison table on the various extract concentrations on the MGT of *P. vulgaris.* (* The mean difference is significant at the .05 level.)

(I) concentration	(J) concentration	Mean Difference (I-J)	Std. Error	Sig.
0%	20%	-.18209(*)	.07668	.019
	40%	-.68317(*)	.07668	.001
	60%	-.94741(*)	.07668	.001
	Gen1	-.39679(*)	.10634	.001
	Gen2	-.66579(*)	.10634	.001
	Gen3	-.73077(*)	.10634	.001
20%	0%	.18209(*)	.07668	.019
	40%	-.50109(*)	.05210	.001
	60%	-.76532(*)	.05210	.001
	Gen1	-.21470(*)	.09023	.019
	Gen2	-.48370(*)	.09023	.001
	Gen3	-.54868(*)	.09023	.001
40%	0%	.68317(*)	.07668	.001
	20%	.50109(*)	.05210	.001
	60%	-.26423(*)	.05210	.001
	Gen1	.28639(*)	.09023	.002
	Gen2	.01738	.09023	.848
	Gen3	-.04760	.09023	.599
60%	0%	.94741(*)	.07668	.001
	20%	.76532(*)	.05210	.001
	40%	.26423(*)	.05210	.001
	Gen1	.55062(*)	.09023	.001
	Gen2	.28161(*)	.09023	.002
	Gen3	.21664(*)	.09023	.018
Gen1	0%	.39679(*)	.10634	.001
	20%	.21470(*)	.09023	.019
	40%	-.28639(*)	.09023	.002
	60%	-.55062(*)	.09023	.001
	Gen2	-.26900(*)	.11649	.023
	Gen3	-.33398(*)	.11649	.005
Gen2	0%	.66579(*)	.10634	.001
	20%	.48370(*)	.09023	.001
	40%	-.01738	.09023	.848
	60%	-.28161(*)	.09023	.002
	Gen1	.26900(*)	.11649	.023
	Gen3	-.06498	.11649	.578
Gen3	0%	.73077(*)	.10634	.001
	20%	.54868(*)	.09023	.001
	40%	.04760	.09023	.599
	60%	-.21664(*)	.09023	.018
	Gen1	.33398(*)	.11649	.005
	Gen2	.06498	.11649	.578

Table 3a; shows the ANOVA table on the MGP and MGT of *C. frutescens* **(ANOVA Table)**

				Sum of Squares	df	Mean Square	F	Sig.
MGP extract	*	Between Groups	(Combined)	2422.349	4	605.587	8.930	.001
		Within Groups		7595.336	112	67.816		
		Total		10017.685	116			
MGT extract	*	Between Groups	(Combined)	5.579	4	1.395	7.130	.001
		Within Groups		21.907	112	.196		
		Total		27.485	116			

Table 3b; the influence of the various extracts on the MGP of *C. frutescens (*LSD)

(I) extract	(J) extract	Mean Difference (I-J)	Std. Error	Sig.
Control	Water E	13.02469(*)	3.06901	.001
	methanol	16.97531(*)	3.06901	.001
	thiophene	14.19753(*)	3.36193	.001
	residue	18.14815(*)	3.36193	.001
WaterE	Control	-13.02469(*)	3.06901	.001
	methanol	3.95062(*)	1.94101	.044
	thiophene	1.17284	2.37724	.623
	residue	5.12346(*)	2.37724	.033
methanol	Control	-16.97531(*)	3.06901	.001
	Water E	-3.95062(*)	1.94101	.044
	thiophene	-2.77778	2.37724	.245
	residue	1.17284	2.37724	.623
thiophene	Control	-14.19753(*)	3.36193	.001
	Water E	-1.17284	2.37724	.623
	methanol	2.77778	2.37724	.245
	residue	3.95062	2.74501	.153
residue	Control	-18.14815(*)	3.36193	.001
	Water E	-5.12346(*)	2.37724	.033
	methanol	-1.17284	2.37724	.623
	thiophene	-3.95062	2.74501	.153

* The mean difference is significant at the .05 level

Table 3c; Multiple comparison table of the extract concentrations on the MGP of *C. frutescens* (Dependent Variable: MGP for *C. frutescens*)

(I) concentration	(J) concentration	Mean Difference (I-J)	Std. Error	Sig.
0%	20%	4.07407(*)	1.51182	.008
	40%	17.48148(*)	1.51182	.001
	60%	22.96296(*)	1.51182	.001
	Gen1	11.11111(*)	2.09652	.001
	Gen2	20.00000(*)	2.09652	.001
	Gen3	23.33333(*)	2.09652	.001
20%	0%	-4.07407(*)	1.51182	.001
	40%	13.40741(*)	1.02708	.001
	60%	18.88889(*)	1.02708	.001
	Gen1	7.03704(*)	1.77896	.001
	Gen2	15.92593(*)	1.77896	.001
	Gen3	19.25926(*)	1.77896	.001
40%	0%	-17.48148(*)	1.51182	.001
	20%	-13.40741(*)	1.02708	.001
	60%	5.48148(*)	1.02708	.001
	Gen1	-6.37037(*)	1.77896	.001
	Gen2	2.51852	1.77896	.160
	Gen3	5.85185(*)	1.77896	.001
60%	0%	-22.96296(*)	1.51182	.001
	20%	-18.88889(*)	1.02708	.001
	40%	-5.48148(*)	1.02708	.001
	Gen1	-11.85185(*)	1.77896	.001
	Gen2	-2.96296	1.77896	.099
	Gen3	.37037	1.77896	.835
Gen1	0%	-11.11111(*)	2.09652	.001
	20%	-7.03704(*)	1.77896	.001
	40%	6.37037(*)	1.77896	.001
	60%	11.85185(*)	1.77896	.001
	Gen2	8.88889(*)	2.29662	.001
	Gen3	12.22222(*)	2.29662	.001
Gen2	0%	-20.00000(*)	2.09652	.001
	20%	-15.92593(*)	1.77896	.001
	40%	-2.51852	1.77896	.160
	60%	2.96296	1.77896	.099
	Gen1	-8.88889(*)	2.29662	.001
	Gen3	3.33333	2.29662	.150
Gen3	0%	-23.33333(*)	2.09652	.001
	20%	-19.25926(*)	1.77896	.001
	40%	-5.85185(*)	1.77896	.001
	60%	-.37037	1.77896	.835
	Gen1	-12.22222(*)	2.29662	.001
	Gen2	-3.33333	2.29662	.150

* The mean difference is significant at the .05 level.

Table 3d; Multiple comparison table of the various extracts on the MGT of *C. frutescens*.

LSD

(I) extract	(J) extract	Mean Difference (I-J)	Std. Error	Sig.
Control	Water E	-.62912(*)	.16482	.001
	methanol	-.68536(*)	.16482	.001
	thiophene	-.60442(*)	.18055	.001
	residue	-.95566(*)	.18055	.001
WaterE	Control	.62912(*)	.16482	.001
	methanol	-.05624	.10424	.591
	thiophene	.02470	.12767	.847
	residue	-.32654(*)	.12767	.012
methanol	Control	.68536(*)	.16482	.001
	Water E	.05624	.10424	.591
	thiophene	.08094	.12767	.527
	residue	-.27030(*)	.12767	.036
thiophene	Control	.60442(*)	.18055	.001
	Water E	-.02470	.12767	.847
	methanol	-.08094	.12767	.527
	residue	-.35124(*)	.14742	.019
residue	Control	.95566(*)	.18055	.001
	Water E	.32654(*)	.12767	.012
	methanol	.27030(*)	.12767	.036
	thiophene	.35124(*)	.14742	.019

* The mean difference is significant at the .05 level

Table 3e Multiple comparison table of the various extract concentrations on the MGT of *C. frutescens* (* the mean difference is significant at the .05 level)

(I) concentration	(J) concentration	Mean Difference (I-J)	Std. Error	Sig.
0%	20%	-.27825(*)	.11163	.014
	40%	-.60697(*)	.11163	.001
	60%	-1.05481(*)	.11163	.001
	Gen1	-.39782(*)	.15481	.012
	Gen2	-1.01169(*)	.15481	.001
	Gen3	-1.45746(*)	.15481	.001
20%	0%	.27825(*)	.11163	.014
	40%	-.32872(*)	.07584	.001
	60%	-.77656(*)	.07584	.001
	Gen1	-.11957	.13136	.365
	Gen2	-.73344(*)	.13136	.001
	Gen3	-1.17922(*)	.13136	.00
40%	0%	.60697(*)	.11163	.001
	20%	.32872(*)	.07584	.001
	60%	-.44785(*)	.07584	.001
	Gen1	.20915	.13136	.114
	Gen2	-.40472(*)	.13136	.003
	Gen3	-.85050(*)	.13136	.001
60%	0%	1.05481(*)	.11163	.001
	20%	.77656(*)	.07584	.001
	40%	.44785(*)	.07584	.001
	Gen1	.65700(*)	.13136	.001
	Gen2	.04312	.13136	.743
	Gen3	-.40265(*)	.13136	.003
Gen1	0%	.39782(*)	.15481	.012
	20%	.11957	.13136	.365
	40%	-.20915	.13136	.114
	60%	-.65700(*)	.13136	.001
	Gen2	-.61387(*)	.16958	.001
	Gen3	-1.05965(*)	.16958	.001
Gen2	0%	1.01169(*)	.15481	.001
	20%	.73344(*)	.13136	.001
	40%	.40472(*)	.13136	.003
	60%	-.04312	.13136	.743
	Gen1	.61387(*)	.16958	.001
	Gen3	-.44577(*)	.16958	.010
Gen3	0%	1.45746(*)	.15481	.001
	20%	1.17922(*)	.13136	.001
	40%	.85050(*)	.13136	.001
	60%	.40265(*)	.13136	.003
	Gen1	1.05965(*)	.16958	.001
	Gen2	.44577(*)	.16958	.010

Table 4a; Extract on the MGP and MGT of *Rorripa indica* **(ANOVA Table)**

				Sum of Squares	df	Mean Square	F	Sig.
MGP extract	*	Between Groups	(Combined)	10362.404	4	2590.601	29.933	.001
		Within Groups		9693.141	112	86.546		
		Total		20055.545	116			
MGT extract	*	Between Groups	(Combined)	15.578	4	3.895	11.704	.001
		Within Groups		37.269	112	.333		
		Total		52.847	116			

Table 4b; Multiple comparison table of the various extracts on the MGP of *R. indica* (LSD)

(I) extract	(J) extract	Mean Difference (I-J)	Std. Error	Sig.
Control	Water E	37.59259(*)	3.46703	.001
	methanol	30.98765(*)	3.46703	.001
	thiophene	27.16049(*)	3.79794	.001
	residue	30.61728(*)	3.79794	.001
Water E	Control	-37.59259(*)	3.46703	.001
	methanol	-6.60494(*)	2.19274	.003
	thiophene	-10.43210(*)	2.68555	.001
	residue	-6.97531(*)	2.68555	.011
methanol	Control	-30.98765(*)	3.46703	.001
	Water E	6.60494(*)	2.19274	.003
	thiophene	-3.82716	2.68555	.157
	residue	-.37037	2.68555	.891
thiophene	Control	-27.16049(*)	3.79794	.001
	Water E	10.43210(*)	2.68555	.001
	methanol	3.82716	2.68555	.157
	residue	3.45679	3.10100	.267
residue	Control	-30.61728(*)	3.79794	.001
	Water E	6.97531(*)	2.68555	.011
	methanol	.37037	2.68555	.891
	thiophene	-3.45679	3.10100	.267

* The mean difference is significant at the .05 level.

Table 4c; Extract concentrations on the MGP of *R. indica* (Dependent Variable: MGP for *R. indica*

(I) concentration	(J) concentration	Mean Difference (I-J)	Std. Error	Sig.
0%	20%	23.53086(*)	2.73933	.001
	40%	34.19753(*)	2.73933	.001
	60%	40.86420(*)	2.73933	.001
	Gen1	21.97531(*)	3.79876	.001
	Gen2	30.49383(*)	3.79876	.001
	Gen3	39.38272(*)	3.79876	.001
20%	0%	-23.53086(*)	2.73933	.001
	40%	10.66667(*)	1.86101	.001
	60%	17.33333(*)	1.86101	.001
	Gen1	-1.55556	3.22336	.630
	Gen2	6.96296(*)	3.22336	.033
	Gen3	15.85185(*)	3.22336	.001
40%	0%	-34.19753(*)	2.73933	.001
	20%	-10.66667(*)	1.86101	.001
	60%	6.66667(*)	1.86101	.001
	Gen1	-12.22222(*)	3.22336	.001
	Gen2	-3.70370	3.22336	.253
	Gen3	5.18519	3.22336	.111
60%	0%	-40.86420(*)	2.73933	.001
	20%	-17.33333(*)	1.86101	.001
	40%	-6.66667(*)	1.86101	.001
	Gen1	-18.88889(*)	3.22336	.001
	Gen2	-10.37037(*)	3.22336	.002
	Gen3	-1.48148	3.22336	.647
Gen1	0%	-21.97531(*)	3.79876	.001
	20%	1.55556	3.22336	.630
	40%	12.22222(*)	3.22336	.001
	60%	18.88889(*)	3.22336	.001
	Gen2	8.51852(*)	4.16133	.043
	Gen3	17.40741(*)	4.16133	.001
Gen2	0%	-30.49383(*)	3.79876	.001
	20%	-6.96296(*)	3.22336	.033
	40%	3.70370	3.22336	.253
	60%	10.37037(*)	3.22336	.002
	Gen1	-8.51852(*)	4.16133	.043
	Gen3	8.88889(*)	4.16133	.035
Gen3	0%	-39.38272(*)	3.79876	.001
	20%	-15.85185(*)	3.22336	.001
	40%	-5.18519	3.22336	.111
	60%	1.48148	3.22336	.647
	Gen1	-17.40741(*)	4.16133	.001
	Gen2	-8.88889(*)	4.16133	.035

* The mean difference is significant at the .05 level

Table 4d; The multiple comparison table on Effect of the various extracts on the MGT of *R. indica.*

(I) extract	(J) extract	Mean Difference (I-J)	Std. Error	Sig.
Control	Water E	-1.44680(*)	.21498	.001
	methanol	-1.16361(*)	.21498	.001
	thiophene	-1.22837(*)	.23550	.001
	residue	-1.00301(*)	.23550	.001
Water E	Control	1.44680(*)	.21498	.001
	methanol	.28319(*)	.13597	.040
	thiophene	.21843	.16652	.192
	residue	.44379(*)	.16652	.009
methanol	Control	1.16361(*)	.21498	.001
	Water E	-.28319(*)	.13597	.040
	thiophene	-.06476	.16652	.698
	residue	.16060	.16652	.337
thiophene	Control	1.22837(*)	.23550	.001
	Water E	-.21843	.16652	.192
	methanol	.06476	.16652	.698
	residue	.22536	.19228	.244
residue	Control	1.00301(*)	.23550	.001
	Water E	-.44379(*)	.16652	.009
	methanol	-.16060	.16652	.337
	thiophene	-.22536	.19228	.244

* The mean difference is significant at the .05 level.

Table 4e Multiple comparison table of the extract concentrations on the MGT of *R. indica*

(I) concentration	(J) concentration	Mean Difference (I-J)	Std. Error	Sig.
0%	20%	-.75327(*)	.15374	.001
	40%	-1.43048(*)	.15374	.001
	60%	-1.68577(*)	.15374	.001
	Gen1	-.35444	.21320	.099
	Gen2	-.82077(*)	.21320	.001
	Gen3	-1.83382(*)	.21320	.001
20%	0%	.75327(*)	.15374	.001
	40%	-.67721(*)	.10445	.001
	60%	-.93249(*)	.10445	.001
	Gen1	.39883(*)	.18091	.030
	Gen2	-.06750	.18091	.710
	Gen3	-1.08055(*)	.18091	.001
40%	0%	1.43048(*)	.15374	.001
	20%	.67721(*)	.10445	.001
	60%	-.25529(*)	.10445	.016
	Gen1	1.07604(*)	.18091	.001
	Gen2	.60970(*)	.18091	.001
	Gen3	-.40334(*)	.18091	.028
60%	0%	1.68577(*)	.15374	.001
	20%	.93249(*)	.10445	.001
	40%	.25529(*)	.10445	.016
	Gen1	1.33132(*)	.18091	.001
	Gen2	.86499(*)	.18091	.001
	Gen3	-.14806	.18091	.415
Gen1	0%	.35444	.21320	.099
	20%	-.39883(*)	.18091	.030
	40%	-1.07604(*)	.18091	.001
	60%	-1.33132(*)	.18091	.001
	Gen2	-.46633(*)	.23355	.048
	Gen3	-1.47938(*)	.23355	.001
Gen2	0%	.82077(*)	.21320	.001
	20%	.06750	.18091	.710
	40%	-.60970(*)	.18091	.001
	60%	-.86499(*)	.18091	.001
	Gen1	.46633(*)	.23355	.048
	Gen3	-1.01305(*)	.23355	.001
Gen3	0%	1.83382(*)	.21320	.001
	20%	1.08055(*)	.18091	.001
	40%	.40334(*)	.18091	.028
	60%	.14806	.18091	.415
	Gen1	1.47938(*)	.23355	.001
	Gen2	1.01305(*)	.23355	.001

* The mean difference is significant at the .05 level

Table 5a; The effect of the extract source on the seedling of the test plants

The results 'indicate that there a significant reduction effect on the root length and shoot length, however the results indicate a non significant effect on the leaf area.

ANOVA		Sum of Squares	df	Mean Square	F	Sig.
Shoot length	Between Groups	57.092	2	28.546	22.981	.001
	Within Groups	592.513	477	1.242		
	Total	649.605	479			
Root length	Between Groups	46.964	2	23.482	24.272	.001
	Within Groups	461.479	477	.967		
	Total	508.443	479			
Leaf area	Between Groups	21.649	2	10.825	.365	.695
	Within Groups	14155.317	477	29.676		
	Total	14176.966	479			

Table 5b; Multiple comparisons on the effects of the extracts from the roots of *T. munita* and *T. patula* on the growth of the roots, shoot and the leave areas of the test plants The LSD= 0.05

Dependent Variable	(I) source	(J) source	Mean Difference (I-J)	Std. Error	Sig.
Shoot length	*T. patula*	*T. minuta*	-.05880	.10725	.584
		Sterile soil	-1.17523*	.17785	.001
	T. minuta	*T. patula*	.05880	.10725	.584
		Sterile soil	-1.11644*	.17785	.001
	Sterile soil	*T. patula*	1.17523*	.17785	.001
		T. minuta	1.11644*	.17785	.001
Root length	*T. patula*	*T. minuta*	-.09120	.09465	.336
		Sterile soil	-1.07824*	.15695	.001
	T. minuta	*T. patula*	.09120	.09465	.336
		Sterile soil	-.98704*	.15695	.001
	Sterile soil	*T. patula*	1.07824*	.15695	.001
		T. minuta	.98704*	.15695	.001
Leaf area	*T. patula*	*T. minuta*	-.09815	.52419	.852
		Sterilesoil	-.73977	.86927	.395
	T. minuta	*T. patula*	.09815	.52419	.852
		Sterile soil	-.64162	.86927	.461
	Sterile soil	*T. patula*	.73977	.86927	.395
		T. minuta	.64162	.86927	.461

*. The mean difference is significant at the 0.05 level.

Table 5c; The effect of the leaf extracts of *T. minuta* and *T. patula* on the shoot and root lengths of the test plant seedlings.

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Rootlength	Between Groups	52.651	3	17.550	18.328	.001
	Within Groups	455.793	476	.958		
	Total	508.443	479			
Shootlength	Between Groups	69.763	3	23.254	19.090	.001
	Within Groups	579.842	476	1.218		
	Total	649.605	479			
Leafarea	Between Groups	20.707	3	6.902	.232	.874
	Within Groups	14156.259	476	29.740		
	Total	14176.966	479			

Table 5d; The post hoc test of comparison using the LSD test for determining contributing factors to the significant reduction in the shoot and root length.

Dependent Variable	(I) location	(J) location	Mean Difference (I-J)	Std. Error	Sig.
Root length	Root	Leaf	.17870	.10527	.090
		control	-1.00324*	.15615	.000
		seedling	-.18102	.13316	.175
	Leaf	Root	-.17870	.10527	.090
		control	-1.18194*	.16309	.000
		seedling	-.35972*	.14124	.011
	control	Root	1.00324*	.15615	.000
		Leaf	1.18194*	.16309	.000
		seedling	.82222*	.18234	.000
	seedling	Root	.18102	.13316	.175
		Leaf	.35972*	.14124	.011
		control	-.82222*	.18234	.000
Shoot length	Root	Leaf	.08171	.11874	.492
		control	-1.18912*	.17612	.000
		seedling	-.42315*	.15019	.005
	Leaf	Root	-.08171	.11874	.492
		control	-1.27083*	.18395	.000
		seedling	-.50486*	.15931	.002
	control	Root	1.18912*	.17612	.000
		Leaf	1.27083*	.18395	.000
		seedling	.76597*	.20566	.000
	seedling	Root	.42315*	.15019	.005
		Leaf	.50486*	.15931	.002
		control	-.76597*	.20566	.000
Leaf area	Root	Leaf	.03310	.58670	.955
		control	-.67620	.87021	.438
		seedling	.02074	.74212	.978
	Leaf	Root	-.03310	.58670	.955
		control	-.70931	.90891	.436
		seedling	-.01236	.78714	.987
	control	Root	.67620	.87021	.438
		Leaf	.70931	.90891	.436
		seedling	.69694	1.01619	.493
	seedling	Root	-.02074	.74212	.978
		Leaf	.01236	.78714	.987
		control	-.69694	1.01619	.493

*. The mean difference is significant at the 0.05 level.

Table 5e; The post hoc test of comparison using the LSD test for determining contributing factors to the significant reduction in the shoot and root length. *. The significant at the 0.05 level.

Dependent variable	(I) extract	(J) extract	Mean Difference (I-J)	Std. Error	Sig.
Rootlength	water	methanol	.05705	.11615	.624
		thiophene	-.17271*	.13882	.004
		residue	-.27271*	.13882	.050
	methanol	water	-.05705	.11615	.624
		thiophene	-.22976	.13882	.099
		residue	-.32976*	.13882	.018
	thiophene	water	.17271	.13882	.214
		methanol	.22976	.13882	.099
		residue	-.10000	.15828	.528
	residue	water	.27271*	.13882	.050
		methanol	.32976*	.13882	.018
		thiophene	.10000	.15828	.528
Shootlength	water	methanol	-.18077	.13021	.166
		thiophene	-.25027	.15563	.108
		residue	-.60266*	.15563	.001
	methanol	water	.18077	.13021	.006
		thiophene	-.06951	.15563	.005
		residue	-.42189*	.15563	.007
	thiophene	water	.25027	.15563	.108
		methanol	.06951	.15563	.655
		residue	-.35238*	.17745	.048
	residue	water	.60266*	.15563	.001
		methanol	.42189*	.15563	.007
		thiophene	.35238*	.17745	.048
Leafarea	water	methanol	-.01513	.61789	.980
		thiophene	-.17921	.73852	.808
		residue	-.07040	.73852	.924
	methanol	water	.01513	.61789	.980
		thiophene	-.16408	.73852	.824
		residue	-.05527	.73852	.940
	thiophene	water	.17921	.73852	.808
		methanol	.16408	.73852	.824
		residue	.10881	.84204	.897
	residue	water	.07040	.73852	.924
		methanol	.05527	.73852	.940
		thiophene	-.10881	.84204	.897