# EFFECTS OF MESQUITE (*Prosopis juliflora* DC.) LEAF EXTRACT AND SOIL FROM UNDERNEATH ITS CANOPY ON GROWTH, YIELD AND YIELD COMPONENTS OF ETHIOPIAN MUSTARD (*Brassica carinata* A.BRAUN)

**M.Sc. THESIS**

**DURETTI ENSARMU BOBASSA**

**MAY 2017**
**HARAMAYA UNIVERSITY, HARAMAYA**

# Effects of Mesquite (*Prosopis juliflora* DC.) Leaf Extract and Soil from Underneath its Canopy on Growth, Yield and Yield Components of Ethiopian Mustard (*Brassica carinata* A.Braun)

**A Thesis Submitted to the Department of Biology, College of Natural and Computational Sciences, Postgraduate Program Directorate**
**HARAMAYA UNIVERSITY**

**In Partial Fulfillment of the Requirements for the Degree of**
**MASTER OF SCIENCE IN BOTANY**

**Duretti Ensarmu Bobassa**

**May 2017**
**Haramaya University, Haramaya**

## HARAMAYA UNIVERSITY

## Postgraduate Program Directorate

We hereby certify that we have read and evaluated this thesis titled **"Effects of Mesquite (*Prosopis juliflora* DC.) Leaf Extract and Soil from underneath its Canopy on Growth, Yield and Yield Components of Ethiopian Mustard (*Brassica carinata* A.Braun)"** prepared under our guidance by Duretti Ensarmu Bobassa. We recommend that the thesis be submitted as fulfilling the thesis requirements.

| | | |
|---|---|---|
| Meseret Chimdessa (PhD, Associate Professor) | ________ | ________ |
| Major Advisor | Signature | Date |
| Sasikumar J.M. **(**PhD, Associate Professor) | ________ | ________ |
| Co-Advisor | Signature | Date |

As members of the Board of Examiners of the M.Sc. Thesis Open Defense Examination, we certify that we have read and evaluated the thesis prepared by Duretti Ensarmu Bobassa and examined the candidate. We recommend that the thesis be accepted as it fulfills the requirements for the Degree of Master of Science in BOTANY.

| | | |
|---|---|---|
| ____________________ | ____________________ | ____________________ |
| Chairperson | Signature | Date |
| ____________________ | ____________________ | ____________________ |
| Internal Examiner | Signature | Date |
| ____________________ | ____________________ | ____________________ |
| External Examiner | Signature | Date |

Final approval and acceptance of the thesis is contingent upon submission of a final copy of the thesis to the Council of Graduate Studies (CGS) through the School Graduate Committee (SGC) of the candidate's major school.

# DEDICATION

This thesis is dedicated to all my beloved parents and friends.

# STATEMENT OF THE AUTHOR

By my signature below, I declare and affirm that this thesis is a genuine result of my own work. I have followed all ethical and technical principles of scholarship in the preparation, data collection, data analysis, and compilation of this thesis. Any scholarly matters that are included in the thesis had been given recognition through citation.

This thesis is submitted to Haramaya University in partial fulfillment of the requirements for the Degree of Master of Science in Botany. The thesis is deposited in the library of Haramaya University to be made available to borrowers under the rules and regulations of the library. I solemnly declare that this thesis has not been submitted to any other institution anywhere for the award of any academic degree, diploma, or certificate.

Brief quotations from this thesis may be made without requiring special permission, if an accurate and complete acknowledgement of the source is made. Requests for permission for extended quotations from or reproduction of this thesis in whole or in part may be granted by the head of the Department of Biology when in his or her judgment, the proposed use of the material is in the interest scholarship. In all other instances, however, permission must be obtained from the author.

Name: Duretti Ensarmu Bobassa                    Signature: _________________

Date May 2017

Department: Biology

# BIOGRAPHICAL SKETCH

The author was born in Iteya Hetosa *Woreda*, Tedo Laman *Keble*, East Arsi, Arsi Zone of Oromia Regional State, Ethiopia from her mother Zeyni Ibso and her father Ensarmu Bobassa in 12/107/995. She attended elementary and secondary school education at Tedo Elementary, Asella Andinat Secondary and Asella preparatory Schools from 2008-2012, respectively. In 2013, she joined Haramaya University and graduated with the Degree of Bachelor of Science in Biology in July 2015. After graduation, she joined the Postgraduate Program at Haramaya University in September 2016 to pursue a study leading to the Degree of Master of Science in BOTANY.

# ACKNOWLEDGMENTS

First, I would like to express my deepest and heartfelt thanks as well as my most sincere appreciation and gratitude to my major advisor Dr. Meseret Chimdessa and my co-adviser Dr. Sasikumar J.M.for their keen interest, dedicated and meticulous supervision, for amicably motivating and scientifically supporting me during the whole period of the study as well as for genuinely and constructively criticizing my work from the time the study was conceived right up to its completion. The visits, they made to my research sites on a number of occasions and the scientific discussion I held with them in the research undertaking were exemplary and worthy of deepest gratitude.

I would also like to thank Ministry of Education for providing me with a funding to pursue the study leading to the Degree of Master of Science, and Haramaya University for facilitating my study.

The kind-hearted treatment and warm welcome offered to me by the staff of the College of Natural and Computational Sciences, particularly the Botany Section of Haramaya University is sincerely appreciated. My sincere gratitude goes to Ato Yimeslal Atnafu, Technical Assistant in the Botany Laboratory and all other biology laboratory Technicians for providing me with the facilities and services required for my study.

Last but not the least; my special thanks are due to my family members especially my husband (Temam Jemal) for giving me the encouragement and moral support to complete this work.

# ACRONYMS AND ABBREVIATIONS

| | |
|---|---|
| ANOVA | Analysis of variance |
| CRD | Completely Randomized Design |
| FAO | Food and Agriculture Organization |
| HU | Haramaya University |
| HURC | Haramaya University Research Center |
| IAS | Invasive Alien Species |
| SOM | Soil Organic Matter |
| SPSS | Statistical Package for Social Sciences |

## TABLE OF CONTENTS

***Continued…***

# LIST OF TABLES

# Effects of Mesquite (*Prosopis juliflora* DC.) Leaf Extract and Soil from Underneath Its Canopy on Growth, Yield and Yield Components of Ethiopian Mustard (*Brassica carinata* A.Braun)

## ABSTRACT

P. juliflora *is known to interfere with the growth of plants growing in its vicinity by the release of allelochemicals during the decomposition of its litter. An attempt was made to determine the effects of different concentrations (0%, 5%, 10%, 15%, and 20%) of* P. juliflora *hexane and ethanol leaf extracts on seedling growth, yield and yield components of* B. carinata*. Soil seedbed and extracts were also prepared from soils sampled from different sites of distance gradient from* P. juliflora *to evaluate the same paramaters under laboratory conditions. Soil has also sampled from different sites in distance gradient from* P. juliflora *to grow* B. carinata *in pots in greenhouse.* P. juliflora *leaf extracts obtained by the two solvents significantly inhibited seed germination and seedling growth in a concentration dependent manner (0%, 5%, 10%, 15%, and 20%). Soil toxicity test conducted through soil-bed growth and soil extract treatment did not have any negative impact on measured parameter(germination percentage, radicle length, plumule length, shoot length and root length, whereas cultivation of* B. carinata *on soils obtained from different points in distance gradient from* P. juliflora *showed significant difference among sampling points(Beneath, edge, 1m and 2m). That is, germination, growth, yield parameters and yield found to increase when cultivated on soil samples away from* P. juliflora *invaded area. Generally, the results revealed that allelochemicals from* P. juliflora *are toxic to* B. carinata *that care must be taken when cultivating it around* P. juliflora*.*

***Keywords:*** Allelochemicals, Brassica carinata, Leaf extract, Prosopis juliflora, Soil properties

# 1. INTRODUCTION

Plant invasions are widely accepted as one of the major threats to global biodiversity across the planet. Massive declines in biodiversity have often been reported in areas where the alien plants have heavily invaded (Cronk and Fuller, 1995; Vitousek *et al*., 1997; Wilcove *et al*., 1998; Pauchard and Shea 2006). The spread of invasive alien species (IAS) is now recognized as one of the greatest threats to the ecological and economic well being of the planet. IAS is causing enormous damage to biodiversity and agricultural system that, we depend on. These alien species outcompete, infect or transmit diseases, compete, hybridize with the native ones or attack them (Wittenberg and Cock, 2001). With increasing trade and globalization, movement of people and goods also increased. This facilitated the spread of IAS.

*P. juliflora*, Fabaceae is a noxious invasive weed that is native to America extending from Peru to Mexico. Currently, it occurs as invasive weed in 25 African countries including Ethiopia (GISP, 2004). *P. juliflora*, which was reported as an aggressive invasive plant species in the world (Pasiecznik *et al*., 2001) shows a wide spectrum of adaptations that enable it to successfully establish in new habitats. *P. juliflora* has been widely distributed in Ethiopia as a biological soil and water conservation agent during the late 1970s. Now it is considered as a major threat because of its invasive nature. *P. juliflora* has an aggressive invasive character invading pastureland, irrigated cultivated lands and irrigation canals causing an irreversible displacement of natural pasture grasses as well as native tree species (Kassahun et *al*., 2004).

In terms of coverage, the areas most adversely affected nationally include the Afar and Somali Regions in the east and southeast of the country and the area around Dire Dawa City. There are also moderately affected areas in Amhara, Oromia, Southern Nations Nationalities and Peoples (SNNP) and Tigray Regions – that is, in the mainly dry lands of Central, East and North Ethiopia (Steele, 2009).

The success of *P. juliflora* is largely attributed to the high number of seeds produced and their efficient dispersal mechanisms (Shiferaw *et al*., 2004). In addition, its fast growing ability, dormant seeds, attractive and rewarding pods, seeds maintaining viability in the droppings of livestock and wild animals, resistance to browsing, incredible ability of re-sprouting and fast coppice growth (Shiferaw *et al*., 2004), and high water use efficiency contribute to its invasion.

Different plant parts, including flowers, leaves, leaf litter and leaf mulch, stems, bark, roots, soil and soil leachates and their derived compounds, can have allelopathic activity that varies over a growing season (Uniyal and Chhetri, 2010). If some of those compounds are released to the environment, from leaching, litter decomposition, root exudation, or direct volatilization, they could affect (either positively or negatively) germination and growth of other species. Allelopathic compounds can be released into the soil by a variety of mechanisms that include decomposition of residues, root exudation, and volatilization (Weston, 2005). It has been noted that the fallen leaves, leachates or root exudates of mesquite release phytotoxic substances that affect all ground vegetation under its canopy (Siddiqui *et al*., 2009).

Phytochemical analysis of mesquite showed that mesquite contains phenolics, tannins, steroids, flavonoids, alkaloids and terpenoids in leaf extracts. Stem contains, steroids, phenolics, flavonoids and terpenes in minimum concentrations, while root has saponin, alkaloids, phenolics, steroids, flavonoids, tannins and terpenes (Singh, 2012). *P. juliflora* is reported to influence the growth of other plants through its allelochemicals (Rizvi and Rizvi, 1992; Thoyabet *et al*., 2009). From this, the folowing hypotheses were made.

I) Tissue extracts of *P.juliflora* would inhibit seed germination, seedling growth of crop plants and

II) II) Leachates from its tissue/decomposed structures would contain allelochemicals that would affect growth and yield of crop plants. In this study, therefore, one of the common vegetable crops, *Brassica carinata* (Brassicaceae) was used as test plant to test the hypotheses.

**General objective:**

To investigate the effects of *P. juliflora* leaf extracts and soils from underneath its canopy on growth, yield and yield components of *B. carinata*.

**Specific objectives:**

- ✓ To measure the impact of *P. juliflora* leaf extracts on *B. carinata* seed germination and seedling growth under laboratory condition.
- ✓ To evaluate the influence of *P. juliflora* soil extract on *B. carinata* seed germination and seedling growth under laboratory condition on.

- ✓ To determine growth, yield and yield components of *B. carinata* grown on soils affected by *P. juliflora* under greenhouse condition.
- ✓ To determine the effects of *P. juliflora* invasion on soil physical and chemical properties.

# 2. LITERATURE REVIEW

## 2.1. Mesquite (*P.juliflora* DC.)

### 2.1.1.Nomenclature, habitat and growth

The genus *Prosopis* belongs to the family Leguminosae (Fabaceae), sub-family Mimosoideae. *Prosopis* contains 44 species, of which 40 are native to the Americas, three to Asia and one to Africa(Burkart, 1976). Burkart (1976) described the *Prosopis* species as trees reaching a height between 3 to 12 metres, which can also appear as a shrub. In all species, the wood is hard and the branches are spread, forming a round or flat-topped crown and green foliage. *Prosopis* trees are evergreen and are deciduous only on very arid sites (Maydell, 1986).

*Prosopis* species are adapted to areas with low rainfall and long periods of drought once they are established and are able to tap groundwater or any other water source during the first years. The lateral roots play an important role during rainy seasons or periods of abundant water, for instance, in irrigated areas. The trees are also able to absorb moisture through their foliage during light rains or from dew or other atmospheric sources of moisture (Pasiecznik *et al*., 2001).

Taxa of *Prosopis* (mesquite; Fabaceae) occur in most of the world's hot arid and semi-arid regions as native or introduced species (Pasiecznik *et al*., 2001). They have been introduced globally and have become naturalized or invasive in many places (Rejmánek and Richardson, 2013). Several *Prosopis* species are also ‘weedy’ in parts of their native ranges (Pasiecznik *et al*., 2001). *P.juliflora* is part of the *P. juliflora-pallida* complex, a taxonomically complicated, interrelated, and controversial group both in the native and non-native ranges of species in this complex (Palacios *et al*., 2012).

### 2.1.2. Global history of introduced *P. juliflora*

*Prosopis* species, some of which are classified as woody weeds, can be classified into two categories: species that occur in their native range or those which have been deliberately introduced, grow, and expand in exotic areas. *Prosopis* is able to survive in the most inhospitable environment, on nutrient poor, saline and alkaline soils, and in hot climates. However, despite their usefulness to humans and animals, *Prosopis* species have shown to be highly invasive, particularly in exotic environments. They are capable of transferring fertile land into dense thickets of impenetrable tree stands (Pasiecznik *et al*., 2001; Pasiecznik *et al*., 2004; Pasiecznik *et al*., 2006a).

Globally, the four major successfully introduced *Prosopis* species are *P. juliflora, P. pallida, P. glandulosa,* and *P. velutina*. The first two species were mainly introduced in tropical regions, while the latter two were introduced in sub-tropical regions. *P. juliflora* has, however, become naturalized in several regions and continents. Introductions of *P. juliflora* have taken place in tropical regions of four continents: the Americas, Australia and the Pacific, Asia (particular India and Pakistan), and Africa including the Middle East. Of all introduced *Prosopis* species, *P. juliflora* performs significantly better than the other *Prosopis* species, especially in the dry tropics. But, it is also the species that is most invasive in exotic environments (Pasiecznik *et al*., 2004).

There are several species of *Prosopis* that have been introduced in Africa and have become noxious weeds. However, only *P. africana* is native to the continent and can be found in Senegal, in regions from Guinea-Bissau to Nigeria, in Cameroon, in Sudan, Uganda and in Ethiopia (Burkhart, 1976). Among the most common species introduced were *P. pubescens*, *P. juliflora*, *P. chilensis*, *P. glandulosa var. glandulosa*, *P. glandulosa var. torreyana* and *P. velutina* (Pasiecznik *et al*., 2006a).

The introduction of *P. juliflora* into Ethiopia took place in the late 1970s in Dire-Dawa, with seeds possibly derived from India. Introductions in the north of the country, in Afar, are believed to have been undertaken by workers of the Middle Awash irrigation project in the late 1970s and early 1980s, with seeds either from Dira-Dawa, from Kenya, or from the Sudan. Meanwhile, *Prosopis* is continuously invading areas of the pastoralists of the Afar and Isa groups in the Afar National Regional State (AfNRS) and has infested areas that are hundreds of kilometre away from its original introductions (Shiferaw *et al*., 2004). Other introductions over large areas in Ethiopia took place as part of the Food for Work Programme, which lasted until 1988. Even today, *Prosopis* species are planted as shade trees and for living fences (Sertse and Pasiecznik, 2005).

In Ethiopia's neighboring country, Eritrea, the introduction of *P. juliflora* probably took place during the 1980s, however, not earlier that the 1970s. It is also not quite clear from where the introductions took place. It is assumed that *P. juliflora* was brought into the country by livestock from the neighbouring country, Sudan. There are close trading links between the Kassala State in the Sudan and the nearby neighbouring towns in Eritrea. Another possible place of entrance of the species into the country is assumed to be from the Afar region of Ethiopia. Meanwhile, *P.juliflora* has become a noxious weed in many of the dry areas of the country (Bokrezion, 2008).

### 2.1.3. Negative effects of *P.juliflora* on rural livelihoods

Mesquite invasion forms impermeable, dense thickets. It reduces grass cover of grazing lands and consequently affects stocking density (Pasiecznik, 1999). The invasion is also a major problem for agricultural lands. Mesquite is accused for diminishing ground water (Pasiecznik, 1999; Pasiecznik *et al*., 2001; Pasiecznik *et al*., 2004) with the help of its long tap root system. The leaves have allelopathic effects inhibiting under canopy growth (Al-Humaid and Warrag, 1998; Nakamo *et al*., 2003); the pollen also causes allergic reactions (Pasiecznik, 1999). The thorns are very poisonous for both humans and animals. These elements enable mesquite to affect the livelihoods of the rural poor.

Why some exotic plants, when introduced to a new part of the world, become far more abundant and have greater impact than in their native range is one of the most puzzling questions in ecology (Callaway and Maron,2006; Callaway *et al*.,2012). *P. juliflora* appears to be one of these species.

### 2.1.4. Invasion promoting Biological and ecological traits of *P. juliflora*

Due to their broad ecological amplitudes, *Prosopis* trees have adapted to a wide range of different types of soil and sites and are able to survive on the poorest lands, which are unsuitable for many other tree species. The tree species can be found on various soil types from pure sandy soils to heavy clay and stony soils. The most abundant *prosopis* trees are found in their native range at altitudes below 200 m above sea level, but they can frequently establish themselves up to 1,500 m above sea level of elevation (Maydell, 1986). Like many other invasive species, *P. juliflora* could also be able to tolerate environmental stress conditions (Kumar and Mathur, 2014), which can drive the species to invade new habitats (Uveges *et al*., 2002; Kercher and Zedler, 2004).

*Prosopis* trees are able to out-compete the indigenous vegetation and spread rapidly, in the worst case becoming agricultural weeds. Geesing *et al.* (2000) suggest that the woody legumes of *Prosopis* species have a competitive advantage on soils with low levels of nitrogen, creating islands of fertility through N fixation. Thus, for instance, under the influence of overgrazing, eroded and degraded poor nitrogen-deficient soils give *Prosopis* seedlings a competitive advantage. The dominant factor that is responsible for the spread of *Prosopis* species is the interaction between climate and land use. Livestock rearing causes a reduction in, or even destroys, herbaceous competition, which is the cause for the decline of soil nitrogen levels. The widespread seed dispersal by livestock creates conditions where the expanding *Prosopis* seed bank only needs to wait for favorable conditions to germinate and establish a new stand (Pasiecznik *et al*., 2001).

*Prosopis* produces a large number of small-size seeds (23 +/- 4 seed/ pod with a mean weight of 0.0275 g), which are adapted for endozoochory (Fenner, 1985). The seeds passing through the guts of animals receive treatments that facilitate germination. The faeces itself probably acts as fertilizer in the critical phase of the establishment of the seedlings. The dispersal of the seeds in the faeces also occurs at some distance from the mother plant, which allows dispersal over a wide terrain (Shiferaw *et al*., 2004).

The dynamic vegetative reproduction, in combination with small seeds and short juvenile periods, are indications that a species will be a strong and competitive invader (Kolar and Lodge, 2001). A short juvenile period, together with the remarkable coppicing ability of resprouting and fast coppice growth from stumped or damaged trees, give *Prosopis* a considerable advantage over other plants in the competition for space (Shiferaw *et al*., 2004). In its native range, densities of *P. juliflora* can be high relative to other leguminous shrubs and trees, but its canopies can have much stronger facilitative effects on neighbors than other leguminous tree species (Larrea Alcazer and Soriano, 2008). Many other *Prosopis* species, in their native ranges, create ‘‘resource islands’’ with higher concentrations of organic matter, nitrogen, phosphorus and potassium beneath their canopies and behave as strong facilitators of other species (Zou *et al*., 2005).

The ecological advantages of *Prosopis* compared with many native species and the resulting invasion of the tree causes a reduction in biodiversity of the native flora. Research has revealed that underneath *Prosopis* canopies, the number of species and the evenness, frequency and density of indigenous species are significantly lower compared to the situation in open space. However, the number of seedlings of those native plants that are found is higher under the *Prosopis* canopy than away from its canopy. The soil fertility also seems to be significantly enhanced beneath *Prosopis* canopies. Such observations have led to the conclusion that allelopathic inhibitions by *Prosopis* are the reason behind the suppressive effect of this species on the indigenous plant biodiversity (El-Keblawy and Al-Rawai, 2007).

### 2.1.5. Invasions and controls of *P. juliflora*

Currently, *P. juliflora* poses a threat to indigenous biodiversity wherever it is established in Ethiopia in general, in the Middle Awash area in particular because of its weedy and invasive nature. In the Middle Awash, about 30,000 hectare of grassland, rangelands, water points and croplands are estimated to be occupied by *P. juliflora* (Mehari, 2008). Areas that are currently invaded by *P. juliflora* were important sources of forage for livestock for the Afar people. The invasion by *P. juliflora* reduces grass availability and stocking density by livestock. It affects the plant biodiversity by creating a physical barrier on seedlings of other plant species, preventing sunlight to reach to the under canopy vegetation, lowering the water table and by releasing various chemicals that may have negative effect on the native plant species.

In numerous tropical arid and semi-arid regions, the genus *Prosopis* has invaded large areas wherever optimal conditions for its spread are available. The adaptive ecological traits of the *Prosopis* species, especially their competitiveness against indigenous plant species, have led to an invasion in some areas that creates hazards for people and the environment. In Ethiopia, *Prosopis* is an aggressive invader of the pastoral lands of the Middle and Upper Awash Valley that causes serious problems for the pastoralists in the region. The tree causes substantial difficulties for the farmers who cultivate irrigated land because of the invasion into the crop fields and the problems it causes to the irrigation system. In the most infested areas, it affects the composition and structure of the indigenous species. *Prosopis* is classified as one of the three most problematic invasive species in the country, together with *Parthenium hysterophorus* L., an agricultural weed, and the Water hyacinth, *Eichhornia crassipes* (Mart.) Solms (Teketay and Bekele, 2002).

From many places in the tropical world where *Prosopis* invasions have caused damage to the environment and to people's livelihood, there are reported experiences of success in controlling such invasions. However, complete eradication of *Prosopis* has proved to be a difficult and expensive undertaking. Although there are reports of successful interventions in smaller areas invaded by the species, it has shown to be extremely difficult, even impossible, to eradicate it over a wide terrain. One of the important requirements necessary to succeed in eradication is the control of the movement of animals in order to hinder new infestations.

### 2.1.6. Allelopathic effects of *P. juliflora*

The chemicals that released from the allelopathic plants are known as allelochemicals. Allelochemicals in majority are secondary metabolites, released into the environment as exudates, volatiles and/or residues of plant tissue decomposition (Weston and Duke, 2003). The structure and concentration of allelochemicals varies with the biological and non-biological cues, therefore, their targets and functions are different (Bais, 2003).

In allelopathy, plant growth is negatively affected due to competition of the two organisms when using the same resources (Burhan and Shaukat, 2000). It has been reported that the plants which release phytotoxic substances, can have both positive and negative effects with the interaction of weeds and crops (Rebaz *et al*., 2001; Shaukat *et al*., 2002). Phytotoxic plants discharge toxic chemicals or secondary plant metabolites, which affect seed germination, cell division, cell elongation, membrane permeability and ion uptake of the nearby plants (Dongre and Singh, 2007). *P. juliflora* plant is considered both invasive and phytotoxic, because it can delay the growth of some nearby plants species by discharging allelochemicals in the environment (Mehar, 2011).

The leaves of *P. juliflora* contain various chemicals including tannins, flavinoids, steroids, hydrocarbons, waxes and alkaloids (Pasiecznik *et al*., 2001). These are known to have effects on the germination and growth of other plant species. Because of this, the plant diversity (both the number of individual plants of a species and the number of species around *P. juliflora*) are affected by the allelechemicals. Low light under *P. juliflora* canopy also make other plant species' survival difficult (Pasiecznik *et al*., 2001).

Allelopathy generally refers to the inhibitory or stimulatory effects of one plant species on other plant species in terms of germination, growth and development (Patil, 2007). The donor plant release allelochemicals into the surrounding environment through leachates, root exudates and volatilization and hence accumulation of allelochemicals causes toxicity affecting crop growth and finally yield (Ahmed and Wardle, 1994).

## 2.2. Ethiopian Mustard

*B. carinata*, commonly known as Ethiopian mustard is an amphi-diploid (an allopolyploid behaving as a diploid) derived from an ancient cross between *B. oleracea*(2N=18) and *B. nigra* (2N=16) (Mabberley, 2008; Stace, 2010; National Genetic Resources Program, 2014) that belong to the family Brassicaceae. Hybridization occurred at least a few thousand years ago, since it has been cultivated in Ethiopia for at least that long (Alemayehu and Becker, 2002; Mnzava and Schippers, 2007). Traditionally, *B. carinata* seeds also called '*Gomenzer*' in Amharic are used to grease clay pan used for baking traditional Ethiopian bread "*Enjera*", to cure certain ailments or stomach upsets and to prepare some beverages. *B. carinata* is an erect, annual herb growing from 30 to 200 cm tall (Alemayehu and Becker, 2002; Mnzava and Schippers, 2007; APD, 2014). It is usually branched with leaves arranged alternately on stems (Mnzava and Schippers, 2007).

*B. carinata* likely originated in Ethiopia a few thousand years ago (Warwick *et al*., 2006; Mnzava and Schippers, 2007). Its exact native distribution is not well understood because it has been cultivated for a long time in Africa; furthermore, it is often confused with *B. juncea* (Mnzava and Schippers, 2007). It is currently cultivated, native, and/or escaping from cultivation in many countries in Africa (Mnzava and Schippers, 2007). "Truly wild types are not known…" (Mnzava and Schippers, 2007). The NGRP (2014) reports *B. carinata* as naturalized in Ethiopia, but because this is where the species is believed to have originated (Warwick *et al*., 2006).

### 2.2.1. Ecology and production status of Ethiopian Mustard

Smallholder farmers often grow *B. carinata* as a backyard crop on humus-rich soils. In some potential areas where Ethiopian mustard is grown in large scale, the crop prefers moderately heavy and well drained soils with pH of 6.5- 7.6 (Nigussie Alemayehu *et al*., 1996). According to the agronomic studies, a seed rate of 10-15 kg/ha with a fertilizer rate of 46/69 Kg/ha of N/P2O5 is optimum depending on the season and planting techniques. *B. carinata* possesses so many desirable traits like high rusticity and adaptability, low chemical input requirement, strong resistance to disease and water shortages, delayed pod shattering or low pod dehiscence even after maturation, simple mechanization, high amount of crop residue and simple insertion in cereal rotation, drought and salt tolerance, pest resistance, high yield and large seed size (Lazzeri and Avino, 2009).

Under normal conditions, *B.carinata* has a vigorous growth even on marginal soils, the stem and leaves are deep green, with anthocyanin in cold periods; leaves are waxy with seeds high in the erucic acid and glucosinolates. Therefore, not commonly used for production of edible oil (Rakow, 2004). Its cultivation so limited, and grown as garden crop around homestead or sparsely mixed with thick crop stands of maize, sorghum, teff and finger millet (Velasco *et al*., 2004). Even though it has been forgotten in the line of research, a basic experimental study on non-traditional use of the crop has given positive results. For example, after transesterification; the oil exhibit physical and chemical properties suited for bio-diesel (Cardone *et al*., 2002). Industrial uses comprise exchange of biomass to bio-energy (Ofori and Becker, 2008; Sabaghnia *et al*., 2010; Sarhad, 2013). In general, *B. carinata* have proven to be one of the few commercially important oilseed plants to respond to biotechnologies quickly (Pandey *et al*., 1999).

### 2.2.2. Economic importance of Ethiopian Mustard

*B. carinata* was categorized as one of the most important traditional East African vegetables in the amount of both crop area and human nutrition together with *B. juncea*, *B.napus* and *B. oleracea.* Unlike the nutritive value of white cabbage, the nutrient composition of these green leaf cabbages is high (Warwick *et al*., 2006).

Ethiopian mustard is traditionally used for many purposes, such as greasing traditional bread making clay pan, curing certain ailments and preparing beverages (Nigussie Alemayehu *et al*., 2001). The oil is also used for cooking in the country, usually adulterated with oils from niger seed or linseed. Besides the utilization of the crop as oil source, Ethiopian mustard is commonly used as a vegetable in Ethiopia. The young leaves and branches are usually used as a side dish after it has been cooked and served with the known local food of Ethiopia, '*Enjera*'. In some southern part of Ethiopia, the leaf is cooked or boiled with meat and served as a main dish to be eaten with the local bread made from '*Enset*' (*Ensete ventricusum*).

The crop has several industrial uses; according to Gonzalez-Garcia *et al*., (2009) bioethanol can be synthesized from *B. carinata* oil using KOH (Potassium hydroxide) as catalyst. In addition, the fatty acid ethyl esters (biodiesel) from *B. carinata* oil were very stable or did not demonstrate rapid increase in peroxide value, acid value, and viscosity with increasing storage time. *B. carinata* can also be used for arsenic intoxicated soil treatment as a biofumignt; the plant has the ability to tolerate arsenic toxicity by accumulating the substance in its leaves like its parent's *B. oleracea* and *B. nigra* (Artus, 2002).

### 2.2.3. Medicinal importance of Ethiopian Mustard

*B. carinata* could also be used in pharmaceuticals, hirudin a pharmaceutical protein commonly used as anticoagulants to prevent thrombosis has been successfully expressed and purified in seeds of *B. napus* and *B. carinata* (Miao *et al*., 2008). For the production of chemical additive, nervonic oils extracted from *B. carinata* can be used as chemical feedstock in a number of industrial applications including polymers and polymer blends.

Research in USA reported that sulforaphane, a component of mustard has been found to inhibit the proliferation of human breast cancer cells. Oral administration of either sulforaphane or its glucosinolate precursor glucoraphanin has been shown to inhibit carcinogen-induced mammary carcinogenesis in rats (Steinmetz and Potter, 1996). Components of mustard have been shown to have antimutagenic, antidiabetic, antifungal, antimicrobial and antioxidant effects (Miao *et al*., 2008). White mustard seed has been shown to inhibit colon cancer formation when added to the diets of both normal and obese rats. *B.campestris* has been shown to inhibit the formation of carcinogen-induced stomach and uterine cancer in mice. According to Steinmetz and Potter (1996), in addition, cruciferous vegetables have also been shown to reduce the risk of gallbladder and urinary bladder cancer and inhibit the proliferation of lung, pancreatic and prostate cancer cells.

# 3. MATERIALS AND METHODS

## 3.1. Description of the Study Area

The experiment was conducted in Haramaya University (HU) Botany Laboratory and green house found in *Rarre* research field. Haramaya University is located at 42 $^0$ 3' E longitudes, 9 $^0$ 26' N latitude at an altitude of 2006 meters above sea level (m. a .s. l) (FAO, 1990). It is 508 km far from Addis Ababa to the east.

## 3.2. Collection of Soil and Plant Samples

The mature plant leaves of *P. juliflora* were collected from the *P. juliflora* trees located around Dire Dawa rural area. Part of the leaf sample collected was freshly washed carefully with distilled water to remove contaminants and air dried in the laboratory at room temperature until preparation of extracts. For soil sampling, four places that serve as replicates having *P. juliflora* individuals with full of canopy coverage, but edges with no *P. juliflora* individuals were selected. Soils from the depth of about 20cm were collected in distance gradient starting from the underneath the canopy of the four individuals of *P. juliflora*, edge of *P.juliflora* canopy, from 1m and 2m distances away from the edge of the canopy. The soil were sampled by laying a 1×1m plot at each sampling point from each corner and center of the plot and made into composite.  All soil samples were sieved through a 2 mm sieve to remove large clods of dirt, roots and other vegetative materials. Parts of the sampled soils were brought to the laboratory for soil residual toxicity bioassay and chemical and physical analyses while the rest were used to cultivate the test plant in pots in green house.

## 3.3. Preparation of Leaf Extracts

Plant leaf sample was extracted with the help of organic solvents (hexane and ethanol) by soaking of 100gm of the leaf powder in 300 ml of organic solvents separately for 24 hours with intermittent stirring with glass rod. Then the extracts were filtered using whatman No 1 filter paper and stored in the refrigerator until bioassay. The extracts were allowed to dry in order to completely remove the solvents under rotary evaporator. The prepared crude extracts were further dissolved in distilled water to have 5%, 10%, 15% and 20% concentrations of leaf extract for treatment group while distilled water used as control (0%) accordingly.

## 3.4. Testing of the Effect of Leaf Extract on Germination of Test Crop

Seeds of *B. carinata* were first surface sterilized using 15% Sodium hypochlorite for 20 min, rinsed and washed with distilled water several times (Tinnin and Kirk-Patrick, 1985). Then 10 seeds were placed evenly on Whatman No.1 filter paper in Petri dishes of 9cm that received 15 ml of 5, 10, 15 or 20% extracts. The negative control received distilled water. The petri-dishes were kept in the laboratory and always kept moist by adding 2ml of extract for treatment group and distilled water control group in equal time interval, and moved round to balance position effect. The experiment was done in a completely randomized design with three replications. Germination percentage, radicle length (cm), plumule length (cm),root length (cm) and shoot length (cm) were recorded over 15 days of incubation.

## 3.5. Effect of Soil Residual Toxicity on Ethiopian Mustard

### 3.5.1. Soil bed bioassay

Soils from the four selected *P. juliflora* were sampled in distance gradient, that is, from underneath *P. juliflora* thickets, at the edge of *P. juliflora* canopy, 1 and 2 m away from the canopy. The soils were then dried at room temperature in laboratory and sieved through 2 mm sieve to remove non-soil materials. One gm of soils from the four treatment sites and four distance gradients were then used as seedbed in petridish by topping them with a single sheet filter paper. The petridishes were moistened with 15 ml of distilled water and seeds of test species were sown and left for 3 days at room temperature. The experiment was done in completely randomized design with three replications. Germination parameters such as germination percent, plumule and radical length were recorded for over 15 days of sowing the seeds.

### 3.5.2. Soil extract bioassay

Five gm of soils from the four treatment sites and four distance gradients were dissolved in 100 ml distilled water and filtered after 24 hrs. Then the soil solution was filtered. The filtrate (15 ml) was used against the same test species as used in the case of leaf extracts in petridishes in the laboratory. Germination parameters such as germination percent, plumule and radical length were recorded for over 15 days of sowing the seeds.

#### 3.5.3. Cultivation of test plant in pots in greenhouse

Seeds (10) of the test plant were sown in pots filled with soils obtained from four sites of selected *P. juliflora* individuals in four-distance gradient as indicated above. Eight pots (two pots having soils from each treatment) were arranged on a table in three replicates in greenhouse. The plants were watered with tap water regularly to keep soils always moist, but were not fertilized. Data collected included germination percentage and growth performance such as shoot length, shoot fresh and dry weights and yield and yield related parameters (Patnaik, 1998).

### 3.6. Analyses of Soil physico-chemical properties

Soil samples were physico-chemically analyzed following standard techniques (Hussain, 1989). Soil pH were determined with a pH meter (pH rex-2 lei-ci, Shanghai) with a 1: 2.5 w/v (soil: distilled water) ratio. Soil organic matters (SOM) were measured using the K2Cr2O7–H2SO4 oxidation method (Nelson and Sommers, 1982). Total phosphorus (P) and available P were determined using the colorimetric Molybdenum-blue-method (Olsen and Sommers, 1982). Total nitrogen was determined using *kjeldahli* digestion method. Each analysis was replicated three times.

### 3.7. Data Analysis

To determine statistical difference between the treatments, data were subjected to one-way ANOVA using statistical package SPSS version 16. Differences between means were considered statistically significant at $P<0.05$.

# 4. RESULTS AND DISCUSSION

## 4.1 Effects of Leaf Extracts on Germination

The study revealed that both ethanol and hexane leaf extracts of *P. juliflora* significantly reduced seed germination parameters (i.e., percent germination, plumule and radicle lengths) at all concentration levels when compared with the control (Table 1). Degree of inhibition found to increase with increasing extract concentration. Percent germination, plumule and radicle lengths were highly negatively affected by hexane extracts than ethanol extracts. That is to say, complete failures of seed germination were observed when treated with hexane extracts at 15 and 20% concentration levels. This may be due to the varying potential of the two solvents to yield different compounds owing to their varying polarity. This result, in general, showed that clearly *P. juliflora* has allelopathic effect on *B.carinata* germination, which is in agreement with the reports of Sundaramoorthy *et al*. (2004) who reported that the *P. juliflora* significantly inhibited the seed germination in Pearl millet. Such inhibitory effects of *P. juliflora* have also been reported previously by other authors. For example, Al-Humaid and Warrag (1998) reported that aqueous leaf extracts of *P. juliflora* showed inhibitory affects on both the radicle and plumule lengths of *Cynodon dactylon.* Similarly, Khan *et al*. (2005) have found that aqueous extract application of *P. juliflora* significantly reduced the germination and seedling growth of wheat. They also indicated that maximum degree of inhibition occurred when leaf extract was used than stem and root extracts.

Table 1. Effect of ethanol and hexane leaf extracts of *P. juliflora* on germination percentage, plumule and radicle lengths

| Extract concentration (%) | Ethanol extract | | | Hexane extract | | |
|---|---|---|---|---|---|---|
| | Germination percentage (%) | Radicle length (cm) | Plumule length (cm) | Germination percentage (%) | Radicle length (cm) | Plumule length (cm) |
| Control (0%) | 89.25±2.17$^{a}$ | 1.0±0.05$^{a}$ | 1.10±0.14a | 89.25±2.17$^{a}$ | 1.0±0.05$^{a}$ | 1.10±0.14$^{a}$ |
| 5% | 78.0±1.68$^{b}$ | 0.74±0.04$^{b}$ | 0.73±0.10$^{b}$ | 77.25±2.43$^{a}$ | 0.68±0.08$^{b}$ | 0.73±0.04$^{b}$ |
| 10% | 75.0±2.20$^{b}$ | 0.54±0.07$^{c}$ | 0.44±0.02$^{c}$ | 58.75±9$^{b}$ | 0.42±0.03$^{c}$ | 0.44±0.05$^{c}$ |
| 15% | 57.5±3.23$^{c}$ | 0.44±0.07$^{c}$ | 0.33±0.04$^{c}$ | - | - | - |
| 20% | 48.75±4.27$^{d}$ | 0.25±0.02$^{d}$ | 0.27±0.03$^{c}$ | - | - | - |

**Note: (-); no germination at all. Differences between means were considered statistically significant at *P<0.05*; Values followed by different small letters in a column are significantly different while those with the same letters are not statistically vary. Each value is a mean of four replicates ± SE (n=4).**

## 4.2 Effects of Leaf Extracts on Shoot and Root Growth

The results of the present finding signify that both ethanol and hexane leaf extracts at all concentration levels were significantly (P< 0.5) reduced shoot and root lengths when compared with the control. Growth was also found to progressively decrease with increasing extract concentrations (Table 2). Nakano *et al*. (2002) previously indicated the growth inhibitory effect of syringin and lariciresinol isolated from *P. juliflora* on lettuce seedlings and Barnyard grass. Aqueous extracts of *P. juliflora* was also reported to reduce seedling growth in *B. campestris.* Leaf aqueous extracts of *P. juliflora* inhibited root and shoot growth of *Cynodon dactylon* (Al-Humaid and Warrag 1998). In other study, the mesquite extract is reported to cause maximum reduction in root length of wheat (Siddiqui *et al*., 2009).

Table 2. Effect of leaf extracts on shoot and root elongation (cm) of *B. carinata*

| Extract concentration (%) | Ethanol extract | | Hexane extract | |
|---|---|---|---|---|
| | Root length (cm) | Shoot length (cm) | Root length (cm) | Shoot length (cm) |
| Control (%) | 1.48±0.14[a] | 1.87±0.4[a] | 1.48±0.14[a] | 1.87±0.36[a] |
| 5% | 1.17±0.21[a] | 1.4±0.2[a] | 1.05±0.11[b] | 1.08±0.10[b] |
| 10% | 0.94±0.19[a] | 1.3±0.3[a] | 0.67±0.09[c] | 0.83±0.12[b] |
| 15% | 0.90±0.25[b] | 1.1±0.2[a] | - | - |
| 20% | 0.56±0.12[b] | 0.6±0.1[b] | - | - |

**Note: (-); no germination at all. Differences between means were considered statistically significant at *P<0.05*; Values followed by different small letters in a column are significantly different while those with the same letters are not statistically vary. Each value is a mean of four replicates ± SE (n=4).**

## 4.3. Effects of Soil Residual Toxicity Bioassay

### a) Soil bed Bioassay

Results of soil-bed culture showed that percent germination, plumule and radicle lengths, and shoot and root growths did not vary significantly when seeds were sown on soils sampled from different points in distance gradient from *P. juliflora* canopy. However, all parameters increase with increasing distance from *P. juliflora*, suggesting that soil toxicity may be higher under *P. juliflora* canopy than far away from it (Table 3). The present results coincide with the findings of Sundaramourty *et al.* (1992) who reported that soil collected from beneath *Acacia tortillus* and *P. cineraria* had no significant inhibition.

Table 3. Effect of soil-bed on seed germination percentage, Plumule and radicle length, and shoot and root length.

| Distance Gradient | Germination percentage (%) | Radicle length (cm) | Plumule length (cm) | Root length (cm) | Shoot length (cm) |
|---|---|---|---|---|---|
| 2m away | 72.5±5.95[a] | 1.04±0.17[a] | 0.81±0.12[a] | 5.25±0.63[a] | 5.29±0.6[a] |
| 1m away | 70±4.56[a] | 0.76±0.19[a] | 0.72±0.1[a] | 4.86±0.19[a] | 4.45±0.26[a] |
| Edge | 67.25±3.07[a] | 0.68±0.12[a] | 0.50±0.12[b] | 4.63±0.38[a] | 4.0±0.1[a] |
| Beneath | 56.25±4.27[b] | 0.44±0.05[b] | 0.46±0.05[b] | 4.16±0.12[a] | 3.53±0.2[b] |

**Note: Beneath, edge, 1m and 2m indicate position of soil sample relative to *P. juliflora.* Differences between means were considered statistically significant at *P<0.05.* Values followed by different small letters in a column are significantly different while those with the same letters are not statistically varied. Each value is a mean of four replicates ± SE (n=4).**

**b) Soil extracts bioassay**

Similarly to soil-bed experiment, the findings of seed germination on extracts of soils sampled from different sites in a distance gradient from *P.juliflora* showed no statistically significant difference (P>0.05) with respect to percent germination, plumule and radicle lengths, and shoot and root growths (Table 4). However, all parameters increased with increasing distance from *P. juliflora*, suggesting that soil toxicity may be higher under *P. juliflora* canopy than far away from it (Table 4).

Table 4. Effect of extracts of soil on seed germination percentage and seedling growth of *B. carinata*

| Distance Gradient | Germination percentage (%) | Radicle length (cm) | Plumule length (cm) | Root length (cm) | Shoot length (cm) |
|---|---|---|---|---|---|
| 2m away | 78.25±5.52[a] | 1.29±0.34[a] | 1.0±0.17[a] | 6.04±0.87[a] | 5.63±0.55[a] |
| 1m away | 75.25±4.39[a] | 0.7±0.07[b] | 0.65±0.12[b] | 4.57±0.22[b] | 4.86±0.19[a] |
| Edge | 71±3.67[a] | 0.65±0.05[b] | 0.62±0.07[b] | 4.32±0.27[b] | 4.63±0.38[a] |
| Beneath | 61.25±4.27[b] | 0.65±0.06[b] | 0.53±0.01[b] | 4.17±0.08[b] | 4.16±0.12[b] |

**Note: Beneath, edge, 1m and 2m indicate position of soil sample relative to *P. juliflora.* Differences between means were considered statistically significant at *P<0.05.* Values followed by different small letters in a column are significantly different while those with the same letters are not statistically varied.Each value is a mean of four replicates ± SE (n=4).**

## 4.4. Effect of Soil Underneath of *P.juliflora* on Cultivations of *B.carinata* in Greenhouse

Germination percentages, plant height, fresh and dry shoot weight, number of pod/plant, number of seed/pod, seed yield/pot and 1000 seeds weight (gm) varied significantly (P<0.05) between soils of different sites in distance gradient from *P. juliflora* (Table 5). That is, all parameters were found to increase with increasing distance from *P.juliflora* canopy. The results of present study is in agreement with the findings of Jones *et al*. (1996) who observed that sorghum grain yields were significantly higher in control (without any trees) and in pruned *P. juliflora* plots than plots underneath *P. juliflora*. This may also suggest that not only underground competitions for water or nutrients are taking place, but also the lack of light due to tree shading may play another important role.

Other studies (e.g., Goel *et al*., 1989; Noor *et al*., 1995; Al Humaid and Warrag, 1998) also indicate that *P. juliflora* has substantial allelopathic potential. Allelochemicals could directly affect plant growth or also influence soil microbes (Inderjit and Putten, 2010). Inderjit *et al*. (2008) compared the effects of soils from the rhizospheres of *P. juliflora* and *P. cineraria* and found that soil under *P. juliflora* had higher concentrations of total phenolics, and inhibited total biomass of *Bambusa arundinacea* more than soil from under the native congener.

Table 5. Impact of Soil on seed germination, growth performance, yield and yield components of *B. carinata*

| Distance Gradient | Beneath of canopy | edge of canopy | 1m away of canopy | 2m away of canopy |
|---|---|---|---|---|
| Germination (%) | 62.5±2.63[c] | 71±2.27[b] | 77.75±2.5[b] | 88.5±1.84[a] |
| plant height (cm) | 77.25±1.11[b] | 92.75±8.37[a] | 99.9±5.0[a] | 106.25±7.2[a] |
| fresh shoot weight (gm) | 12.75±0.75[b] | 15±2.42[b] | 29.5±7.42[a] | 34±5.18[a] |
| dry shoot weight (gm) | 10.25±0.85[b] | 14.5±1.84[b] | 16.75±0.85[a] | 21.25±2.1[a] |
| 1000 seed weight(gm) | 1.36±0.12[d] | 2.24±0.25[c] | 2.85±0.12[b] | 3.92±0.23[a] |
| Number of pod/plant | 23.25±1.18[b] | 31.75±1.18[a] | 33.75±2.4[a] | 38.75±4.23[a] |
| Number of seed/pod | 7.0±0.37[c] | 8.0±0.41[c] | 9.18±0.28[b] | 12.50±0.29[a] |
| Total yield/pot(gm) | 2.41±0.23[c] | 3.52±0.21[b] | 3.75±0.9[b] | 4.75±0.32[a] |

**Note: Differences between means were considered statistically significant at *P<0.05.* Values followed by different small letters in a row are significantly different while those with the same letters are not statistically varied. Each value is a mean of three replicates ± SE (n=3).**

## 4.5. Soil physical and chemical properties

The pH value and available P were showed significant difference between soils sampled from different points in distance gradient from *P. juliflora* (Table 6). Values were found to increase with increasing distance from *P. juliflora*.  However, SOM, total N and total P did not show significance difference (P>0.05) though their values appeared to decrease with increasing distance from *P. juliflora*. The slight acidity of the soil within the canopy zone could be attributed to leaches and exudes from litter fall and roots. The findings of this study were in agreement with those of Bhatia *et al*. (1998) who observed a significant reduction in the soil reaction (pH) under the canopies of *P. juliflora.* Acid soils (generally defined as having a pH<5) present several indirect challenges to plant health, mainly phosphorus (P) deficiency and manganese (Mn) toxicity. The accumulation of total nitrogen under the *P. juliflora* canopy may be partly due to the earlier seasonality of litter fall and reduced leaching under the tree canopy. These results corroborate with that of Wasonga (2001) who states the existence of higher concentration of total carbon and nitrogen in the soils within the canopy than in soils in the adjacent open area.

The lower available phosphorus content under canopy in this study may be due to biological processes that are continuously taking place between the Rhizobium bacteria and the tree roots since *P. juliflora* is leguminous tree. Rhizobium bacteria utilize phosphorus in synthesizing their proteins and hence the low level of it under the canopies. Young (1989) observed low phosphorus in sub canopy zones and attributed it to being utilized in biological nitrogen fixation by the Rhizobium bacteria. Low P availability is an inherent challenge to root growth in acid subsoil. Unlike Ca, P moves readily in the phloem and provided to growing root tips by the plant. However, bioavailable P is concentrated in the topsoil because of the low mobility of P in soil and the accumulated effects of biomass deposition and greater microbiological activity in the topsoil (Lynch and Brown, 2001). In contrary, soil organic matter (SOM) accumulates/increases under canopy because of carbon fixation in photosynthesis (Bhojvaid and Timmer, 1998) that this organic matter is transferred into the soil by leaf litter and root activity of *P. juliflora*.

Table 6. Chemical analyses of soil samples that were taken from the P. juliflora invaded sites and the adjacent natural sites

| Soil distance gradient | pH value | SOM (%) | available P (mg/kg) | Total P% | Total N% |
|---|---|---|---|---|---|
| Beneath of canopy | $4.5\pm0.29^b$ | $1.6\pm0.06^a$ | $12.08\pm0.93^b$ | $0.73\pm0.32^a$ | $0.79\pm0.26^a$ |
| Edge of canopy | $5.23\pm0.50^b$ | $1.41\pm0.28^a$ | $15.74\pm0.63^b$ | $0.41\pm0.2^b$ | $0.62\pm0.21^a$ |
| Out of canopy (1-2m) away | $6.61\pm0.31^a$ | $1.33\pm0.15^a$ | $19.88\pm1.52^a$ | $0.28\pm0.24^b$ | $0.14\pm0.03^b$ |

**Note: Differences between means were considered statistically significant at *$P<0.05$*. Values followed by different small letters in a column are significantly different while those with the same letters are not statistically varied.Each value is a mean of three replicates (n=3).**

# 5 SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

## 5.1 Summary and Conclusions

This study was conducted with the objective of evaluating the growth inhibitory effects of leaf extracts of *P. juliflora* and soil invaded by it on *B. carinata*. The experiments were done both in the laboratory and greenhouse. Laboratory experiment was done using ethanol and hexane leaf extracts (5, 10, 15 and 20%) on germination and seedling growth of *B. carinata.* Soil toxicity was also tested on the same parameters under laboratory condition while greenhouse experiment was pot culture of *B. carinata* on soils obtained from different points distance gradient from *P. juliflora* canopy. The results showed that both solvents extracts of *P. juliflora* inhibited germination and growth of seedlings under laboratory conditions in a concentration dependent manner. However, soil residual toxicity test made in the laboratory showed no significant negative effect on germination and growth of seedlings. However, pot cultivation of *B. carinata* in greenhouse showed that soils obtained far from *P. juliflora* canopy favored all measured parameters. In conclusion, Leaf extracts and soils invaded by *P. juliflora* have allelopathic effect on *B. carinata*.

## 5.2 Recommendations

Based on the current results of the finding the following recommendations are forwarded:

- *B. carinata* should not be cultivated on a soil that has been highly occupied by *P. juliflor*a and near by it.
- Similar experiment should be carried out using root and stem of *P. juliflora* with different solvents.

# 6. REFERENCES

Ahmed, M and D.A. Wardle. 1994. Allelopathic potential of vegetative and flowering ragwort (*Senecio jacobaeo*.L) plant against associated pasture species. *Plant and Soil*, 164: 61-68.

Alemayehu, N., and H. Becker. 2002. Genotypic diversity and patterns of variation in a germplasm material of Ethiopian mustard (*Brassica carinata* A. Braun). *Genetic Resources and Crop Evolution*, 49(6):573-582.

Al-Humaid, A.I. and Warrag, M.O.A. 1998. Allelopathic effects of mesquite (*Prosopis juliflora*) foliage on seed germination and seedling growth of bermudagrass (*Cynodon dactylon*). *Journal ofArid Environment*, 38:237-243.

Patil, C.K. 2007. Allelopathic effect of botanicals on major weeds of onion (*Alium cepa* L.). M.Sc. Thesis submitted to the University of Agricultural Sciences, Dharwad, India.

APD (African Plants Database). 2014. African Plants Database (APD), version 3.4.0. Conservatoire et Jardin botaniques de la Ville de Genève and South African National Biodiversity Institute. http://www.ville ge.ch/musinfo/bd/cjb/africa. (Archived at PERAL).

Artus, N. 2002. Effect of arsenate treatment on growth and arsenic accumulation in four high biomass members of the *Brassicaceae*. *In*: The 9th New *Phytologist Symposium*; *Biology Department*, West Chester University, West Chester, USA. pp 193 – 195.

Bais, H. P. 2003. Allelopathy and exotic plant invasion: from molecules and genes to species interactions. *Science*, 301, 1377-1380.

Bhatia, N.P., Adholeya, A. and Sharma, A. 1998. Biomass production and changes in soil productivity during long-term cultivation of *Prosopis juliflora* (Swartz) DC. Inoculated with VA mycorrhiza and Rhizobium spp in semi-arid wasteland. *Biology and Fertility of Soils*, 26: 208-214.

Bhojvaid, P. P. and Timmer, V.R. 1998. Soil dynamics in an age sequence of *Prosopis juliflora* planted for sodic soil restoration in India. *Forest Ecology and Management*, 106(2-3): 181-193. doi: 10.1016/S0378-1127(97)00310-1.

Bokrezion, H. 2008. The ecological and socio-economic role of *Prosopis juliflora* in Eritrea. *Academic Dissertation*, Johannes Gutenberg-Universität Mainz, Germany. p. 218.

Burhan, N. and Shaukat, S.S. 2000. Effects of atrazine and phenolic compounds on germination and seedling growth of some crop plants. *Pakistan Journal Biological Science*, 3: 269-274.

Burkart, A. 1976. A monograph of the genus *Prosopis* (Leguminosae subfamily Mimosoideae). Part 1 and 2. Catalogue of the recognised species of *Prosopis*. *Journal of the Arnold Arboretum*, 57:219–249. 450–526.

Callaway, R.M. and Maron, J.L. 2006. What have exotic plant invasions taught us over the past 20 years? *Trends of Ecology and Evolution* 21: 369–374.

Callaway, R.M., Schaffner, U., Thelen, G.C., Khamraev, A. and Juginisov ,T. 2012. Impact of Acroptilon repens on co occurring native plants is greater in the invader's non native range. *Biological Invasion*, 14: 1143–1155.

Cardone, M., Prati, M.V. V.Rocco, Seggiani, M., Senatore, A. and S. Vitolo. 2002. *B.carinata* as an alternative oil crop for the production of bio-diesel in Italy: engine performance and regulated and unregulated exhaust emissions. *Environmental Science and Technology*, 36: 4656 - 4662.

Cronk, Q. C. B. and J. L., Fuller. 1995. Plant Invaders: the "Threats to Natural Ecosystems". Chapman and Hall, London, United Kingdom.

Dongre, P.N. and Singh, A.K. 2007. Inhibition effects of weeds on growth of wheat seedlings. *Journal of Allelopathy*, 20(2): 387-394.

El-Keblawy, A. and Al-Rawai, A. 2007. Effects of salinity, temperature and light on germination of invasive *P. juliflora* (Sw.) D.C. *Journal of Arid Environments*, 61: 555 565.

FAO (Food and agricultural organization). 1990. Food and agricultural organization of the United Nations Agro meteorology group, Remote sensing Center Research and Technology Division. Rome, Italy.

Fenner, M. 1985. Seed ecology. Chapman & Hall, London, UK. 151 p.

Geesing, D., Felker, P. and Bingham, R.L. 2000. Influence of mesquite (*Prosopis glandulosa*) on soil nitrogen and carbon development: Implications for agroforestry and global carbon sequestration. *Journal of Arid Environment*, 46: 157-180

GISP (Global Invasive Species Program). 2004. Africa invaded: The growing danger of invasive alien species. Global Invasive Species Program, Cape Town.

Goel, U., Saxena, D.B. and Kumar, B. 1989. Comparative study of allelopathy as exhibited by *P. juliflora* swartz and *P. cineraria* (L) Druce. *Journal of Chemical and Ecology*, 15: 591–600.

Gonzalez García, S., Gasol, C.M., Gabarrell, X., Rieradevall, J., Moreira, M.T. and Feijoo. G. 2009. "Environmental aspects of ethanol-based fuels from *B. carinata*: A case study of second generation ethanol". *Renewable and Sustainable Energy Reviews*, 13: 2613-2620.

Hussain, F. 1989. Field and Laboratory Manual of Plant Ecology. NAHE, UGC, Islamabad.

Inderjit and van der Putten, W.H. 2010. Impacts of soil microbial communities on exotic plant invasion. *Trends of Ecological Evolution*, 25: 512–519.

Inderjit, Seastedt, T.R., Callaway, R.M., Pollock, J.and Kaur, J. 2008. Allelopathy and plant invasions: traditional, congeneric, and biogeographical approaches. *Biological Invasion*, 10: 875–890.

Jones, M., Sinclair, F.L. and Grime, V.L. 1996: Effect of tree species and crown pruning on root length and soil water content in semi-arid agroforestry. *Plant and Soil*, 201(2): 197-207: doi: 10.1023/A:1004324616942.

Kassahun, Z., Yohannes, L. and Olani, N. 2004. *Prosopis juliflora*: Potentials and Problems. *Arem*, 6: 1-10.

Kercher, S. M. and J. B., Zedler. 2004. Multiple disturbances accelerate invasion of reed canary grass (*Phalaris arundinacea* L.) in a mesocosm study. *Oecologia*, 138: 455-464.

Khan, M.A., Marwat, K.B., Hassan G and Hussain Z. 2005. Bioherbicidal effects of tree extracts on seed germination and growth of crops and weeds. *Pakistan Journal of Weed Science Research*, 11(3-4): 89 – 94.

Kolar, C.S. and Lodge, D.M. 2001. Progress in invasion biology. *Trends of Ecology and Evolution*, 16: 199-204.

Kumar, S. and M., Mathur. 2014. Impact of invasion by *Prosopis juliflora* on plant communities in arid grazing lands. *Tropical Ecology*, 55: 33-46.

Larrea Alcazar, D.M. and Soriano, P.J. 2008. Columnar cacti shrub relationships in an Andean semiarid valley in Western Venezuela. *Plant Ecology*, 196: 153–161.

Lazzeri, L. and Avino, D. L. 2009. Green Chemistry: an agricultural production chain for biolubricants. *In*: workshop on Green Chemistry Future and its Possible Impact on Agriculture, the Whole Farm Tractability Approach . Ispara , Italy.

Lynch, J.P., Brown, K.M. 2001. Topsoil foraging an architectural adaptation of plants to low phosphorus availability. *Plant and Soil*, 237, 225–237.

Mabberley, D. J. 2008. Mabberley's Plant-Book: A Portable Dictionary of Plants, Their Classification and Uses (third edition). Cambridge University Press, New York. pp. 1021.

Maydell, von, J.H. 1986. Trees and shrubs of the Sahel. Their characteristics and uses. Deutsche Gesellschaft für Technische Zusammenarbeit (GTZ). Eschborn, Germany. p. 523.

Mehar, K.S. 2011. Assessment of effect of *Prosopis juliflora* litter extract on seed germination and growth of rice. *Food Science and Quality Management*, 2: 9-19.

Mehari, Z. 2008. Invasion of *Prosopis juliflora* (SW.) DC and Rural Livelihoods. The Case of Afar Pastoralists at Middle Awash Area of Ethiopia. MSc. Thesis, Norwegian University of Life Science, Oslo, pp.3.

Mnzava, N. A. and R. R., Schippers. 2007. *Brassica carinata* A. Braun.: Internet record from Plant Resources of Tropical Africa (PROTA), Online Database. Wageningen University. http://www.prota4u.org/search.asp. (Archived at PERAL).

Nakamo, H., Nakajima, E., Yoshiharu, F., Yamada, K., Shigemori, H., and Hasegawa, K. 2003. Leaching of the allelopathic substance, L-trypatophan from the foliage of mesquite (Prosopis juliflora (Sw.) DC.) plants by water spraying. *Plant Growth Regulators*, 40, 49-52.

Nakano, H., Fujji, Y., Yamada, K., Kosemura, S., Yamamura, S., Hasewaga, K. and Suzuki, T. 2002. Isolation and identification of plant growth inhibitors as candidate(s) for allelopathic substance(s) from aqueous leachate from mesquite (*Prosopis juliflora* (Sw) DC) leaves. *Plant Growth Regulators*, 37:113-117.

Nelson, D.W. and L.E. Sommers.1996. Total Carbon, Organic Carbon and Organic Matter. *SSSA*, *Methods of Soil Analysis*, Part 3; Chemical Methods, p 983.

NGRP (National Genetic Resources Program). 2014. Germplasm Resources Information Network (GRIN). United States Department of Agriculture, Agricultural Research Service, National Genetic Resources Program. http://www.ars-grin.gov/cgibin/ npgs/html/index.pl?language. (Archived at PERAL).

Nigussie Alemayehu and Becker, H.C. 2001. Variation and inheritance of erucic acid content in *Brassica carinata* germplasm collections from Ethiopia. *Plant Breeding*, 120:331-335.

Nigussie Alemayhu, Adefris Teklewold and Tadele Zerihun. 1996. Effect of agronomic practices on seed and oil yields of Ethiopian mustard (*Brassica carinata* A. Braun and rapeseed (*B. napus* L.). *Tropical Agriculture*, 73:94-99.

Noor, M., Salam, U., Khan, M.A. 1995. Allelopathic effects of *P. juliflora* Swartz. *Journal of Arid Environment*, 31: 83–90.

Ofori, A. and H.C. Becker. 2008. Breeding of *Brassica rapa* for biogas production: heterosis and combining ability of biomass yield. *Bioenergy Research*, 1: 98–104.

Olsen,S.R. and L.E. Sommers. 1982. Phosphorus. p. 403-430. *In*: A.L. Page et al. (eds.), *Methods of soil analysis*, *Part II*, *2$^{nd}$ edition*, *Agronomy Monographs* no. 9.

Palacios, R.A., Burghardt, A.D., Frras Hernandez, J.T., Olalde Portugal, V. and Grados, N. 2012. Comparative study (AFLP and morphology) of three species of *Prosopis* of the Section Algarobia: *P. juliflora*, *P. pallida*, *and P. limensis*. Evidence for resolution of the ''*P. pallida–P. juliflora* complex''. *Plant Systematic Evolution*, 298: 165–171.

Pandey, I., Singh, B. and Sachan, J. 1999. *Brassica* hybrid research in India: status and prospects. 10th rapeseed international congress. Pantanagar, India.

Pasiecznik, N. H., P. Felker, P. J. C. Harris, L. N. Harsh, G. Cruz, J. C. Tewari, K. Cadoret and L. J. Maldonado. 2001. The *Prosopis juliflora-Prosopis pallida* Complex. HDRA, Coventry, United Kingdom.

Pasiecznik, N. M. 1999. *Prosopis* pest or providence, weed or wonder tree*?* (Newsletter No. 28): *European Tropical Forest research Network.*

Pasiecznik, N.M., Harris, P.J.C. and Smith, S.J. 2004. Identifying tropical *Prosopis* species: a field guide. HDRA, Coventry, United Kingdom. p.30. Available online: http://www.gardenorganic.org.uk/pdfs/international programme/Identifying*Prosopis* Guide.pdf (Accessed: 15.01.2017)

Pasiecznik, N.M., Harris, P.J.C., Trenchard, E.J. and Smith, S.J. 2006a. The dilemma of *Prosopis*: is there a rose between the thorns? *Biocontrol News and Information*, 27(1): 1N-26N. Available online: http://www.cabi.org/bni/uploads/File/BNI/GN2701.pdf (Accessed: 15.02.2017)

Patnaik, P. 1998. Allelopathic effect of *Eucalyptus* leaves on Ragi (*Finger millet*) crop. Ph.D. Thesis, Berhampur University, Berhampur, Orissa, India.

Pauchard, A. and K. Shea. 2006. Integrating the study of nonnative plant invasions across spatial scales. *Biological Invasions*, 8: 399-413.

Rakow, G. 2004. Species origin and economic importance of *Brassica. Biotechnology in Agriculture and Forestry*, 54: 3-7.

Rebaz, Z, Shaukat, S.S. and Siddiqui, I.A. 2001. Allelopathic potential of *Anagallis arvensis*: A cosmopolitan weed. *Pakistan Journal of Biological Science*, 4: 446-450.

Rejmanek, M., Richardson, D.M. 2013. Trees and shrubs as invasive alien species update of the global database. *Diversity and Distributions*, 2013; 19:1093–1094.

Rizvi, S.J.H. and Rizvi, V. 1992. *Allelopathy: Basic and applied aspects*. Chapman and Hall, London.

Sabaghnia, N., H. Dehghani, B. Alizadeh and M. Mohghaddam. 2010. Heterosis and combining ability analysis for oil yield and its components in rapeseed. *Australian Journal of Crop Science*, 4(6): 390-397.

Sarhad. 2013. Correlations studies for some Agronomic and Quality traits in *Brassica Napus*. L genotypes .*International Journal of plant breeding and Genetics*, 97: 831-840.

Sertse, D. and Pasiecznik, N.M. 2005. Controlling the spread of *Prosopis* in Ethiopia by its utilisation. HDRA, Coventry, United Kingdom.

Shaukat, S.S., Siddiqui, I.A., Khan, G.H. and Zaki, M.J. 2002. Nematacidal and allelopathic potential of *Aregemon mexicana,* a tropical weed. *Plant and Soil*, 245: 239-247.

Shiferaw, H., Teketay, D., Nemomissa, S. and Assefa, F. 2004. Some biological characteristics that foster the invasion of *Prosopis juliflora* (Sw.) DC. at Middle Awash Rift Valley Area, northeastern Ethiopia. *Journal of Arid Environments*, 58: 134–153, http://dx.doi.org/10.1016/j.jaridenv.2003.08.011.

Siddiqui, S., Bhardwaj, S., Khan, S.S. and Meghvanshi, M.K. 2009. Allelopathic effect of different concentration of water extract of leaf of *Prosopis juliflora* on seed germination and radicle length of wheat (*Triticum aestivum* Var-Lok-1). *American European Journal of Science and Research*, 4 (2): 81-84.

Singh, S. 2012. Phytochemical analysis of different parts of *Prosopis juliflora*. *International Journal of Current Pharmaceutical Research*, 4(3): 59-61.

Stace, C. 2010. New Flora of the British Isles (3rd ed.). Cambridge University Press, Cambridge, United Kingdom. pp. 1130.

Steele, P., Breithaupt, J., and Labrada, R. 2009, April. Increased food security: control and management of *Prosopis*. In Proceedings of an Expert Consultation, 4, Awash (Ethiopia), 15-19 October 2007. FAO.

Steinmetz, K. A. and J. D .Potter. 1996.Vegetables, fruits and cancer prevention: A review. *Journal of the American Dietetic Assocaition*, 96:1027–1039.

Sundaromourty, S., N. Kalra and D.N. Sen. 1992. Allelopathic potential of *Acacia tortilis* on seed germination and seedling growth of some legumes. *In*: *Allelopathy in agroecosystems (Agriculture and Forestory)*. (Eds.): P. Tauro and S.S. Narwal. Proceeding First National Symposium, CCS Haryana Agriculture University, India.

Sundramoorthy, S., N. Kalra and D.D., Chawan. 2004. Allelopathy and *Prosopis juliflora* provenance Israel in semi arid agroforestry systems. *Indian Journal of Forest*, 18 (3): 214-220.

Teketay, D. and Bekele, T. 2002. State of forest and forestry in Ethiopia. Working Report Series: Indicators and Tools for Restoration and Sustainable Management of Forests in East Africa. I-TOO Working paper No. 1. p. 56.

Thoyabet, S.A., Ray, R.H., Sven, V. and George, S.E. 2009. Effects of leaf extract of *Zizyphus spinachristi* and *Prosopis juliflora* on each other's seedlings roots. *Allelopathy Journal*, 23: 111-118.

Tinnin, R.O. and Kirkpatrick, L.A.1985. The allelopathic influence of broadleaf trees and shrubs on seedling of *Douglas fir*. *Forest Science*, 31: 945-952.

Uniyal, A.K. ,and Chhetri , S. 2010. An Assessment of Phytotoxic Potential of Promising Agroforestry Treeson Germination and Growth Pattern of Traditional Field Crops of Sikkim Himalaya, India. *American Eurasian Journal of Agriculture and Environmental Science*, 9 (1): 70-78.

Uveges, J. L., A. L. Corbett and T. K. Mal. 2002. Effects of lead contamination on the growth of *Lythrum salicaria* (purple loosestrife). *Environmental Pollution*, 120: 319-323.

Velasco, L.,Nabloussi.,A, Haro.,A.D. and Fernandez-martinez, J. 2004. Allelic variation in linoleinic acid content of high erucic acid Ethiopian Mustard and 59 incorporation of the low linoleinic acid trait into zero erucic acid germplasm. *Plant breeding*, 123: 137 - 143.

Vitousek, P. M., C. M. D'Antonio, L. L. Loope, M. Rejmánek and R. Westbrooks. 1997. Introduced species: a significant component of human-caused global change. *New Zealand Journal of Ecology*, 21: 1-16.

Warwick, S. I., R. K. Gugel, T. Mc Donald, and K. C. Falk, 2006. Genetic variation of Ethiopian mustard (*Brassica carinata* A. Braun) germplasm in western Canada. *Genetic Resources and Crop Evolution*, 53(2):297-312.

Wasonga, V. O. 2001. Effect of *Balanites glabra* canopy cover on soil organic matter, soil moisture and selected grass species production in Isinya Kajiado, Kenya. M.sc. Thesis, University of Nairobi. Kenya.

Weston, L.A. 2005 . History and Current Trends in the Use of Allelopathy for Weed Management. *Cornell University Turfgrass Times*, 13: 529-534.

Weston, L.A. and Duke, S.O. 2003. Weed and crop allelopathy. *Critical Reviews in Plant Science*, 22: 367-389. doi: 10.1080/713610861.

Wilcove, D. S., D. Rothstein, J. Dubow, A. Philips and E. Loso, 1998. Quantifying threats to imperiled species in the United States. *Bioscience*, 48: 607-615.

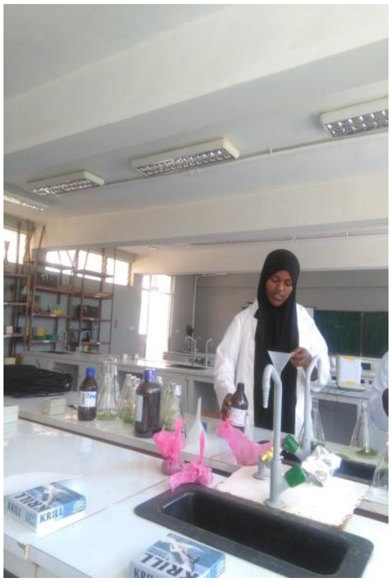
Wittenberg, R, Cock, M.J.W. 2001. Invasive alien species. How to address one of the greatest threats to biodiversity: A toolkit of best prevention and management practices. CAB International, Wallingford, http://dx.doi.org/10.1079/9780851995694.0000.

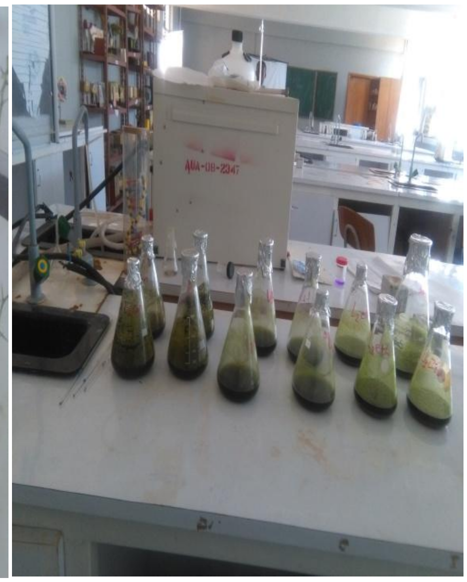
Young, A. 1989. *Agroforestry for soil management 2nd Edition*. CAB-ICRAF, Nairobi.

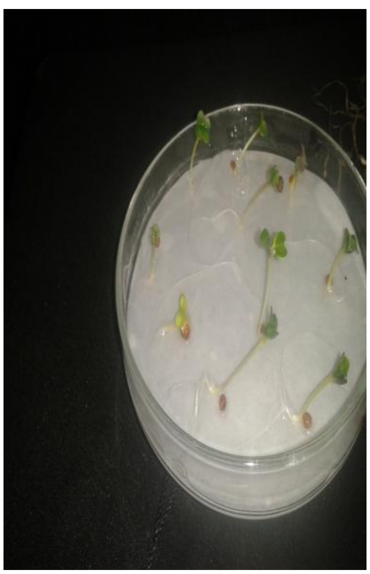
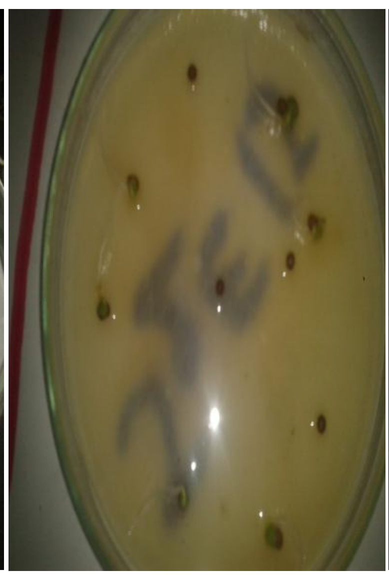
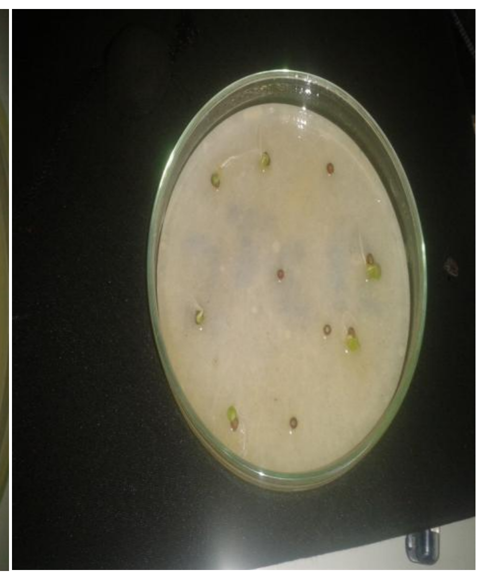
Zou, C.B., Barnes, P.W., Archer, S.and McMurty, C.R. 2005. Soil moisture redistribution as a mechanism of facilitation in savanna tree-shrub clusters. *Oecologia*, 145: 32– 40.

# 7. APPENDIX

## 7.1. Some Figures During Laboratory Work





**Control Treatment** **Hexane Treatment** **Ethanol Treatment**

## 7.2. Some Figures During Greenhouse Work