

**PHYTOTOXICITY OF *Lantana camara* L. EXTRACTS AND SOIL  
FROM BENEATH ITS CANOPY ON CABBAGE (*Brassica carinata* A.)**

**M.Sc. THESIS**

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Canopy on Cabbage (*Brassica carinata*)**

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## **DEDICATION**

To all my lovely family members who have always supported, motivated and inspired my post graduate studies despite many ups and downs.

## STATEMENT OF THE AUTHOR

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## ABBREVIATIONS/ACRONYMS

BLC	Beneath Lantana Canopy
IAS	Invasive Alien Species
IBC	Institute of Biodiversity Conservation
IWCU	International World Conservation Union
OELC	On the Edge of <i>Lantana</i> Canopy
OMLC	One Meter away from <i>Lantana</i> Canopy
TMLC	Two Meters away from <i>Lantana</i> Canopy

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## **Phytotoxicity of *Lantana camara* Extracts and Soil From Beneath its Canopy on Cabbage (*Brassica carinata* )**

### **Abstract**

*Lantana camara* is an invasive weed species that has allelopathic effects on crops and other indigenous associated plants. Allelochemicals from such plants may also have positive or negative impact on soils. Therefore, the research was carried out to investigate the allelopathic effect of leaf extracts of *Lantana camara* and soils invaded by it on *Brassica carinata*. Leaf extracts of *Lantana camara* were prepared with water, hexane and methanol, while distilled water alone was used as control to evaluate germination parameters and growth of seedlings of *Brassica carinata* under laboratory condition. Soil seed-bed and extracts were also prepared from soils sampled from different sites of distance gradient from *Lantana camara* to evaluate germination parameters and growth parameters of *Brassica carinata*. Similarly, soils sampled from different sites in distance gradient from *Lantana camara* were also used to grow *Brassica carinata* in greenhouse. Results showed that water extracts negatively affected germination and growth parameters under laboratory conditions ( $p < 0.05$ ). Comparison between three solvents extracts impact showed that water extract was superior to hexane and methanol extract in negatively affecting performance of the test plant. However, seed germination percent was significantly reduced by soil extracts sampled from two meters away from lantana thicket ( $p > 0.05$ ). Soil pH was found to decrease with increasing distance from beneath lantana canopy. Available phosphorus and total nitrogen were relatively higher in soils collected from 1 and 2 m away from lantana canopy. In greenhouse experiment significant difference was observed between soils of different distance gradient from *Lantana camara* in seed yield of *B. carinata*. Overall, results of this study showed that *Brassica carinata* is sensitive to allelochemicals from *Lantana camara* and it is not advisable to cultivate in areas with huge decomposition of *Lantana camara*.

**Key words:** allelochemicals, Aqueous, *Lantana camara*, Germination

## 1. INTRODUCTION

In nature plant species interact and influence growth and survival of each other. These interactions could be through competition for limited resources or allelopathy (Anderson *et al.*, 1978). There were numerous reports on toxicity of *Lantana camara* to crops (Wilson, 1968). Allelopathy is an interference mechanism in which live or dead plant materials release chemical substances. These chemicals may be released from leaves, stem, root, flowers or fruits, and inhibit the germination and a growth of neighboring plant species.

*Lantana camara* is one of the most invasive alien plant species which is widely distributed. It produces and releases several secondary metabolites (allelochemicals) such as phenolic acids, flavonoids, terpenes, quinines and essential oils, etc (Maiti *et al.*, 2008). Allelochemicals (inhibitors) are produced by plants as by-products and metabolites and released to the environment through many ways (e.g., residue decomposition, root exudation or leaching and volatilization). Allelochemicals inhibit the growth of other plants (Sharma *et al.*, 1998; Duizy *et al.*, 2001). Due to its strong allelopathic properties, fast growth, unpalatable nature, and production of allelochemicals, *Lantana camara* has the potential to interrupt regeneration process of other species by decreasing germination, reducing early growth rates and selectively increasing mortality of other plant species. These cause shifts in species distribution and the increase of dominance by certain annual and perennial weeds in a variety of agro ecosystems, which results in a decline of species or reduction of species diversity (Sharma *et al.*, 2005).

Allelopathic effect of *Lantana camara* results in severe reductions in seedling germination of nearly all species under its cover. Zero growth or only stunted growth has been observed for other species growing close to *Lantana camara*. Allelochemicals promote or inhibit the plant growth based on their concentration, which increases from roots, stem to leaf, making the leaf toxic to grazing animals (Achhireddy and Singh, 1984; Achhireddy *et al.*, 1985). Allelopathy plays an important role in weed-weed interactions (Newman *et al.*, 1975) and weed-crop interactions. Such interactions may lead to the reduction or elimination of associated plants. Furthermore, interference among plants often leads to a superiority of a particular species and failure of the other under natural conditions.

*Lantana camara* is able to colonize large areas, because seed dispersal is via birds and due to its ability to produce allelochemicals which causes it to out compete other plants. It grows mainly in non-arable terrestrial habitats and farmers are reluctant to control it because it is not a threat to crop production. However, it has been reported that the leaves and unripe berries of lantana can produce high levels of poisonous alkaloids which are toxic to livestock and humans. Some of the allelochemicals which have been found in lantana leaves are p-OH-benzoic acid, p-coumaric acid, caffeic acid, vallic acid, syringic acid and gentisic acid (Hussein *et al.*, 2011).

*Lantana camara* is a small thorny flowering shrub with square stems. It is also popular as ornamental plant that consists of many varieties with numerous flower colors such as purple, orange, yellow, red, white and pink. *Lantana camara* releases a very unpleasant aroma and is allelopathic against other plants. The toxins in *Lantana camara* include the triterpene acids, lantadeneA (rehmannic acid), lantadene B and their reduced forms. The essential oil extract of the leaves is effective as insecticide (Bouda *et. al.*, 2001). The effects of weeds on crops can be manifested in agro-ecosystems through dominant competitive position for growth prerequisites such as light, water, and mineral. On the other hand, allelopathic plants inhibit or suppress germination, growth, development or metabolism of crops due to secretion of allelochemicals to the rhizosphere of neighboring crop plants (Altieri, and Doll, 1978).

The competitive and invasive nature of *Lantana camara* stems originate from two primary defenses: allelopathy and resistance to herbivory. Allelochemicals produced in the roots and stems have been shown to negatively influence the growth and competitive ability of surrounding species and eventually to decrease biodiversity (Foy and Inderjit, 2001). This allows for monotypic stands to readily develop, and, once established, it is highly persistent in the environment. Persistence of lantana is due to many factors, but one in particular is its resistance to herbivory due to toxin accumulation in the leaves (Ghisalberti, 2000).

The diverse and broad geographic distribution of *Lantana camara* is a reflection of its wide ecological tolerance. It occurs in diverse habitat and on a variety of soil types. *Lantana* is a perennial shrub generally that grows best in open, un-shaded conditions such as wastelands, the edges of rain forests, beachfronts, agricultural areas, grasslands, riparian zones, scrub/shrub lands, urban areas, wetlands and forests recovering from fire or

logging. Roadsides, railway tracks and canal banks are favored by the species. The plant is found at altitudes from sea level up to 2,000 m and can thrive very well under rainfall ranging from 750 to 5000 mm per annum and it grows up to 3 m height. *Lantana* does not invade intact rain forests, but is found on their margins where natural forests have been disturbed through logging creating gaps and encroaches in the gaps. Further, logging aggravates the condition and allows *lantana* to spread or become thicker in its growth. It cannot survive under dense, intact canopies of taller native forest species (Lonare *et al.*, 2012).

Ethiopia is one of the countries affected by invasive plant species, which have been clearly identified as one of the emerging problems facing the country. A national stakeholder workshop conducted in August 2002 identified and prioritized Invasive Alien Species (IAS) in Ethiopia to be *Prosopijuliflora*, *Parthenium hysterophorus*, *Striga spp.*, *Eichhornia crassipes*, *Lantana camara* and *Acacia spp.* Prioritization of IAS was done by considering facts such as the magnitude of invasiveness, threats to local biodiversity, socioeconomic and health impacts.

*Lantana camara* has aggressively invaded eastern part of Hararge, particularly Babile. People also grow this species as hedge and live fence around their farm land. Though there were reports on the allelopathic effect of *Lantana camara* on some plant species, no detailed study has been conducted on crop plants grown in the area and their decomposition products on the quality of the soil of this area. Therefore, the research was initiated to study the allelopathic effect of leaf extracts of *Lantana camara* and its impact on soils invaded by it with the following objectives.

**General objectives**

- to investigate the allelopathic effect of *Lantana camara* on *Brassica carinata* and the quality of soil invaded by it.

**Specific objectives were:**

- to examine the allelopathic effects of *Lantana camara* leaf extracts (water, hexane and methanol ) on seed germination and seedling growth of *Brassica carinata*
- to assess the effects of *Lantana camara* invasion on soil physical and chemical properties.

## 2. LITERATURE REVIEW

### 2.1 Origin of *Lantana camara*

*Lantana camara* has established and spread in its introduced range worldwide at a cost of native species and habitats so much that the international world conservation union (IWCU) considers it to be among the world's 100 most invasive species. *Lantana camara* is considered as a weed of international significance because of its wide spread distribution in temperate, subtropical and tropical climates and impacts on primary production and biodiversity. The diverse and broad geographic distribution of lantana is a reflection of its wide ecological tolerances. *Lantana camara* typically occurs where there is a moderate to high summer rainfall and well-drained sloping sites, mainly preferring fertile organic soils, but also capable of surviving on siliceous sands and sandstone-derived soils of moderate depth if other conditions, especially year-round moisture, were suitable. *Lantana* does not tolerate water logging, salinity, prolonged drought, dense shading by over storey species, frequent or severe frosts, or winter temperatures with prolonged periods below 5° C (Lowe *et al.*, 2004). It generally grows best in open field situations such as wastelands, rainforest edges, beachfronts, and forests recovering from fire or logging (Sharma *et al.*, 2005).

### 2.2 Distribution of *Lantana camara*

*Lantana camara* is a floriferous, prickly, thicket forming shrub, which is thought to have arisen through deliberate horticultural and natural hybridization, selection and somatic mutation from a number of similar, and probably closely related, central and South American species (Swarbrick *et al.*, 1995). It is native to tropical and subtropical America and has been dispersed throughout the world. As a popular ornamental and hedge plant, several varieties (cultivars) of *Lantana camara* have been widely distributed throughout the tropics, subtropics and warm temperate regions of the world. Following its worldwide introduction, it has become naturalized in some 50 countries and is rated as one of the world's worst weeds (Day *et al.*, 2003).

*Lantana camara* readily invades disturbed sites and communities and so various types of habitats were susceptible to *Lantana camara* invasion. In communities with a naturally dense canopy, *Lantana camara* colonization may be heavily dependent on, and limited to, disturbance zones, edges, and canopy breaks. There is a strong correlation between *Lantana* establishment and disturbance ( Stock, 2005).

In Africa it is also wide spread South of the Sahara Desert and particularly severe in South Africa, Kenya, Uganda, Tanzania, Zambia, Zimbabwe, and Mozambique. It also occurs in Ghana, Nigeria and Angola. *Lantana* is first introduced into South Africa via Cape Town and Kwa Zulu-Natal in 1883. In South Africa it is presently naturalized in the warm, moist subtropical and temperate regions (Stirton, 1977) of the Northern Province, Gauteng, Mpumalanga and KwaZulu-Natal, as well as the southern coastal regions of the Eastern and Western Cape Provinces (Day *et al.*, 2003). *Lantana camara* is currently rated the fourth most wide spread invasive alien plant in South Africa occupying some 2.2 million hectares of forest and plantation margins, watercourses and savannas where it outcompetes and replaces other native vegetation (Simelane, 2002).

In Ethiopia, *Lantana camara* is an exotic species and it has wide ecological tolerance and growing successfully in various soil types (Day *et al.*, 2003). The wide spread nature of this plant is facilitated by moving of seeds through running water, dispersal by birds and animals after eating and excreting undigested seeds (IBC, 2009). Some of the hotspot areas of *Lantana* in Ethiopia include Debre Zeit, Dire Dawa, Hararghe and Somali region. Allelopathic effects of *Lantana camara* on some agricultural crops are given worldwide emphasis, but such scientific investigations are limited in Ethiopia. The agrarian society of Ethiopia is growing many agricultural food crops in order to fulfill the demands of growing population. The dominated agricultural crops in the country are maize, *Zea mays* and wheat, *Triticum turgidum*. Therefore, allelopathic effects of *Lantana camara* leaf powder on germination and growth of *Zea mays* and *Triticum turgidum* was evaluated using pot culture experiment and reported. In the problem areas *Lantana* quickly takes over valuable grazing lands and its dense growth suppresses grasses and other useful forages. In these areas farmers do not want to invest on the control, as it is not a problem in their crop fields. Hence, introduction and release of the already tested and used biological control agents (Taye, 2007) should be a priority in *Lantana* weed management in Ethiopia.

### **2.3. Botanical Aspects of *Lantana camara***

*Lantana camara* is notorious weed and a popular ornamental garden plant belonging to the family verbenaceae, genus *Lantana* and species *Lantana camara*. It is a perennial, summer-growing low erect or subscandent, vigorous shrub with stout recurved prickles and a strong aromatic odor when crushed. It is normally 2-4 m tall, but is capable of becoming a climber reaching up to 10 m in height which is a heavily branched shrub that can grow as compact clumps, dense thickets and as a scrambling and climbing vine. *Lantana camara* grows at altitudes from sea-level to 2000 m.a.s.l tropical, subtropical and temperate climates, with mean annual rainfall of 1000–4000 mm (Binggeli, 2003) and can successfully survive such high levels of rainfall only if the soil is well drained, since it has a tendency to rot in boggy or hydromorphic soils. It also tolerates some shade, growing in plantations and open eucalypt forests, but it does not flower readily under these conditions (Wells and Stirton, 1988; Day *et al.*, 2003).

### **2.4. Invasive Characteristics of *Lantana camara***

*Lantana camara* receives the highest prioritization score based on criteria such as its invasiveness, spatial characteristics, potential impact, potential for control and the conflict of interests with the economic and informal sectors that arises from controlling the weed. The weed invades forests, riverbanks, mountain slopes and valleys, creek banks, fence lines, grassland, and is common in open and semi-open plant communities such as road verges, wood lands and commercial forests where it forms impenetrable stands that obstruct access and utilization. It aggressively pioneers invasion of disturbed ecosystems, tending to crowd out indigenous flora and suppress natural vegetation, thus reducing the carrying capacity of veldt (Robertson *et al.*, 2003).

*Lantana camara*, a globally invasive and highly variable species complex, is one of the most ecologically and economically harmful invasive alien plants of the tropical, subtropical and warm temperate regions. The shrub invades natural ecosystems, where they transform the indigenous vegetation into impenetrable thickets of lantana, which diminish natural pasturage, plantations, reduce biodiversity and devalue the land (Day *et al.*, 2003).

The species has been associated with negative impacts in agriculture where it occurs along riparian areas—decreased water quality and obstruction to accessing water sources. *Lantana camara* is a major weed in many regions of the tropics where it invades natural and agricultural ecosystems (Thomas and Ellison, 1999).

*Lantana* invades disturbed sites, especially open sunny areas such as roadsides, cultivated pastures, fence lines and the edges of forests, but it does not flower as well under a heavy canopy as it is not very shade tolerant. Further, logging aggravates the condition and allows *lantana* to spread or become thicker in its growth. It cannot survive under dense, intact canopies of taller native forest species (Spies & Duplessis, 1987).

Through allelopathic suppression of indigenous plant species, *Lantana* invasions also interrupt regeneration processes and reduce the biodiversity of natural ecosystems (Gentle and Duggin, 1997). Under conditions of high light, soil moisture and soil nutrients *Lantana* is a very efficient competitor against native species. The wide distribution of *lantana camara* indicates a severe threat for the ecosystems whereas homeostatic fitness and phenotypic plasticity also increase the invasiveness of *Lantana camara*. Homeostatic fitness describes the ability of individual or population to maintain relatively constant fitness over a range of environments while phenotypic plasticity is the ability of genotype to modify its growth and development in response to changes in environment (Sharma *et al.*, 2005).

Indirect changes in floral composition occur as a result of modifications of factors such as the physical and chemical properties of soil, changes in competitive potential and light availability through the loss or introduction of new plants (Clegg, 1999).

## **2.5 General Effects of *Lantana camara***

### **2.5.1 Effects on native plant species**

*Lantana camara* dense thickets reduce biodiversity through dominating understory and excluding native species through smothering, reducing their germination and allelopathic effects (Simelane, 2002; Sharma *et al.*, 2005). It is a very effective competitor with native colonizers and is capable of interrupting the regeneration processes of other indigenous species by reducing germination, reducing early growth rates, and increasing mortality (Sharma *et al.*, 2005).

It competes strongly for moisture and nutrients thereby reducing productivity of the native plants. Invasive plants have a multitude of impacts on plant communities through their direct and indirect effects on soil chemistry and ecosystem functions. Plants modify their environment in many ways that include the alteration of some of the basic aspects of the physical and chemical substrates in which they grow through exudates that affect soil structure and mobilize and or cheated nutrients.

*Lantana* invasion significantly alters native species compositions of heavily invaded sites comprising different species composition than both non-invaded and moderately invaded sites (Day *et al.*, 2003). Species composition and structure is altered indirectly through modified soil environment and directly through allelopathic means. Changes in environmental conditions generally lead to an increase in certain species at the expense of others.

### **2.5.2 Effects on soil**

Many exotic invasive plant species may alter nutrient cycles in ways that differ from resident natives. These invasive plant species have high specific leaf areas, growth rate and leaf nutrient concentrations relative to the natives at the same sites and these traits have the potential to increase rates of decomposition and nutrient cycling (Allison and Vitousek, 2004).

Nutrient dynamics may become altered as a result of changes in the physical properties of the soil caused by the introduction of an alien species such as *Lantana camara*, but it is not always the case that soil properties would be altered following alien species invasion (Ehrenfeld, 2003). In dense stands of *Lantana*, the capacity of the soil to absorb rain is lower than under good grass cover. This could potentially increase the amount of run-off and the subsequent risk of soil erosion in areas infested with the weed (Day *et al.*, 2003). Most changes in species composition reflect changes in soil water and nutrient availability, and in turn, changes in availability of essential plant resources such as light, nutrients and water may result in a change of the nature vegetation community (Clegg, 1999).

*Lantana camara* also has a negative effect on soil water supply (Hiremath and Sundaran, 2005). Because the allelochemicals produced by *Lantana camara* could alter the populations of soil microbial symbionts necessary for the early establishment of certain seedlings (Viranjicetal, 2000).

Wide spread of *Lantana camara* infestations regularly impact agriculture, the environment, forest management, recreation and transport. This indicates that the plants can affect soil structure (particle aggregation) by creating pores in soil (Angers and Caron 1998). Plants decrease the bulk density of soils through root channeling and litter deposition, thus making it easier for water and roots to penetrate (Tiedemann and Klemmedson, 1986; Joffre and Rambal 1993).

## **2.6 Management of *Lantana camara***

Mechanical, chemical and biological control methods have been employed to control *Lantana camara* in different parts of the worldwide. Mechanical control can be achieved by physical removal of *Lantana camara* from the beginning, accompanied by planting fast growing species that increase the shade of disturbed sites to prevent the reestablishment of the weed. In practice, mechanical control involves cutting, digging or uprooting, hand pulling and chopping. Although the method is very effective, it is labor intensive (Thomas and Ellison, 2000). Mechanical control is only effective for small infestations and is contingent on continuous and meticulous follow-up. This method minimizes disturbance to nearby vegetation and is effective in killing the plants, but is only feasible where the plants were small, and where they occur in small, isolated clumps (Day *et al.*, 2003).

Chemical control method refers to the control of target invasive alien plants using herbicides (Ezc, 2007). Chemicals were effective, but expensive method for *Lantana camara* control and effectiveness depends on plant size, time of application, mode of application, and the use of surfactants (Stock, 2005). Various herbicide treatments were used and were said to be most effective when applied as a foliar spray or to the base of the stems and cut stumps. Some herbicides were reportedly more effective on particular forms of *Lantana*, with the most effective belonging to the phenoxy acid, benzoic acid, and pyridine groups (Swarbrick *et al.*, 1995).

Another control option for weedy invasive plants is biological control using various insect natural enemies and pathogens. This method is very different from both mechanical and chemical control as it involves the suppression of weeds by insects and microorganisms that feed on the target plant or otherwise parasitize it (Day *et al.*, 2003).

Biological control provides a long-term and cost-effective means of controlling weeds, as agents may be self-sustaining following establishment. Unlike herbicidal application, biological control is environmentally safe and agents were specific to the target weed.

Agents were slow acting and thus inhibit the formation of large empty areas where other invaders can establish. Biological control of alien plants does have disadvantages. The importation, host-specificity testing and eventual release of an insect agent is time-consuming and expensive. However, the aim of biological control is not eradication, rather to reduce the target weed population to below an ecologically or economically determined threshold (Van wilgen*et al.*, 2004).

### 3. MATERIALS AND METHODS

#### 3.1. Description of the Study area

The study was conducted in botany laboratory and *Rarre* green house, in Haramaya University (HU). Haramaya University is located between latitudes of 9° 24' 53.13"N and 9° 24' 51.34"N, and between longitudes of 42° 01'55.69"E and 42° 01' 56.62"E at a distance of 508 km to the East of Addis Ababa. The altitude in the campus varies from 1980 to 2000 meter above sea level.

#### 3.2. Plant materials and Soils

*Lantana camara* leaves and soils affected by it were collected from sites supporting thickets of *L. camara* in Haramaya university campus or its surroundings. For this, aggregates of *Lantana camara* individuals forming 100% canopy cover thickets of approximately equal girth were selected from four sites to serve as replication sites. Fresh leaves of *Lantana camara* were collected from these four sites into paper bags and air-dried in the lab. Soil samples were also taken from these four sites in distance gradient starting from beneath *Lantana camara* canopy, edge of *Lantana camara* canopy, and away from the edge of *Lantana camara* canopy at 1 and 2m distances along a line transect on which a 0.5 X 0.5m plots were laid. Soils composite had been systematically sampled from the upper 20 cm from the center and four corners of the plot and brought in plastic bags to dry in air in the lab. Seeds of the test plant (*Brassica carinata*) were obtained from local market.

#### 3.3. *Lantana camara* Leaf Extraction

Air dried leaves were powdered by using mortar and pestle. Five gram of the powder was macerated in 100ml of distilled water or organic solvents (methanol and hexane) and left on the table in the lab for 24 hours with intermittent stirring with glass rod. After 24 hours of extraction, extract was filtered under suction using Whatmann no 1 filter paper and made to dry in order to completely remove the solvents under rotary evaporator and stored in refrigerator until bioassay.

### **3.4. Experimental Design and Treatments**

#### **3.4.1. *Lantana camara* Leaf Extract Phytotoxicity Bioassay**

The design of the experiment was completely randomized design with three replications. To investigate the impact of extracts on germination of the test plant, the dried extracts obtained using the three different solvents viz. distilled water, methanol and hexane were first dissolved in distilled water separately to get % (w/v) of extract concentration. Then 10 seeds of the test plants were put on filter paper that received 15ml of each extract or distilled water (control) in Petri-dishes of 9cm diameter. The Petri-dishes were arranged on tables in the lab and allow to germinate fully. Petri-dishes were made to get more or less the same amount and type of light throughout incubation. After days of incubation, days to complete germination, percent germination, plumule and radical lengths were determined.

#### **3.4.2. Phytotoxicity and quality of soils invaded by *Lantana camara***

In order to investigate *Lantana camara* (LC) soil residual toxicity on test plants and soil quality, i.e., chemical and physical features of soils affected by *Lantana camara*, soils were sampled in four distance gradient from four *Lantana camara* individuals. For this, places that serve as replicates (at least 4 replicates) having LC individuals with 100% canopy coverage. Then four treatment sites: the site beneath the *Lantana camara* canopy, on the edge of *Lantana camara* canopy, one meter away from *Lantana camara* canopy and two meters away from *Lantana camara* canopy were considered for soil sampling. After that, a plot of 0.5x 0.5m was laid beneath the canopy of *Lantana camara* (selected individuals of *Lantana camara* in 4 replicates) and soil (upper 20cm) were taken from of each the four corners of the plot and the center of the plot and made into composite. Similar plots were laid at the edge of the canopy and then at 1 and 2m distances from the canopy edge to have a distance gradient. Part of the sampled soils were brought to the lab 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 as described below.

### **3.4.3. Determination of *Lantana camara* soil residual toxicity on test plants**

Soil residual toxicity was determined using soil bed and soil extract bioassay experiments as follows.

#### **a. Soil bed bioassay:**

One gram of soils sampled from the four treatment sites in distance gradient as indicated above were spread in Petri dishes to serve as seed-beds and topped with a single sheet of filter paper. The Petri dishes were moistened with 15ml of distilled water and seeds of test species were placed on filter papers and incubated at room temperature.

**b. Soil extract bioassay:** Five gram of soils from four treatment sites were dissolved in 100ml of distilled water and filtered after 24 hrs. The filtrate was used against the same Test species as used in the case of tissue extracts in Petri dishes. For both bioassay types, soils were sieved through 2mm sieve to remove non-soil materials. Data to be collected include all those considered under leaf extract bioassay.

### **3.5. Use of *Lantana* invaded soils for cultivation of test plant**

Seeds (10) of the test plant were sown in pots filled with soils obtained from four sites of *Lantana* thickets in four distance gradient as indicated above. Eight pots (2 pots from each treatment) were arranged on a table in 5 replicates in greenhouse. In order to maintain soil moisture, plants were watered with tap water regularly. Data collected included germination percentage, days to start and complete germination and growth performance.

### **3.6. Analyses of physical and chemical properties of soils invaded by *Lantana camara***

Soils samples were analyzed following the methods described in Page *et al.* (1996). Soil pH was determined with a pH meter with a 1:2.5 w/v (soil: distilled water) ratio. Soil organic matter (SOM) was measured using the oxidation method (Nelson and Sommers, 1982). Total N was measured after Kjeldahl sample preparation and analysis. Total phosphorus (P) and available P were determined using the colorimetric Molybdenum-blue-method (Olsen and Sommers, 1982). Each analysis was replicated four times.

### **3.7. Statistical Analysis**

Analysis of variance (ANOVA) was performed using SAS version 9.1.

## 4. RESULTS AND DISCUSSIONS

### 4.1. Effects of *L. camara* leaf extracts on seed germination of *Brassica carinata*

The seed germination percentage, plumule and radical lengths were reduced by all the solvent extracts when compared with control groups (Table 1). Of the three extract, water extract showed highest inhibitory effect than hexane and methanol extracts. In other related researchers (Jabeen and ahmed, 2009; Hossain and Alam, 2010) suggest that *Lantana camara* leaf extracts have allelopathic effects on germination and behavior of agricultural crops, like *Triticum aestivum* and *Cucurbita pepo*. According to Narwal (1996) and Hossain and Alam (2010) *Lantana camara* has allelopathic property and inhibits the seedling growth of other plants due to the presence of secondary compounds such as phenolic acids and some other phytotoxic chemicals released from the decomposition of leaf litter and roots. *Lantana camara*. Leaf extracts have also been reported to negatively affect germination of agricultural crops such as *Triticum aestivum* and *Cucurbita pepo* (Alam, 2010). However, water extracts showed more inhibition than organic solvent extracts. This may be suggesting that *Lantana camara* contains a significant source of water soluble allelochemical growth inhibitors.

**Table 1. Effect of *Lantana camara* leaf extracts on germination parameters of seeds of *Brassica carinata*.**

Treatments	Germination Parameters		
	Percent Germination rate (%)	Plumule length (cm)	Radicle length (cm)
Control	97±1.53 <sup>a</sup>	2.64 ± 0.16a	18.17 ± 0.19a
HE	83±2.13 <sup>a</sup>	2.40 ± 0.08a	1.75 ± 0.18c
WE	46±5.62 <sup>b</sup>	1.28 ± 0.16b	2.50 ± 0.11c
ME	82±2.91 <sup>a</sup>	2.00 ± 0.06a	10.70 ± 0.13b

HE =Hexane extract, WE = Water extract, ME = Methanol extract

## 4.2. Effects of soils invaded by *Lantana camara* on seed germination of *Brassica carinata*

### 4.2.1. Effect of soil extracts

The accumulated organic matter mulches the soil surface under the invasive species thereby inhibiting the leaching of nutrients from the soil surface. This may be the possible explanation for the higher concentrations of nutrients in the invaded site than un-invaded ones and which can promote germination, plumule and radicle length of the *Brassica carinata* as the results were observed in table (2). The findings of the present study agrees with those of Fan *et al*, (2010) and Sharma and Roghubanshi (2011) who recorded higher concentrations of Mg, Ca and K following *Lantana camara* invasion in Southern China and India respectively.

**Table 2. Germination parameters of seeds of *Brassica carinata* treated with soil extracts sampled from *Lantana camara* canopy and away from *Lantana camara* in distance gradient.**

Treatments	Germination rate (%)	Plumule length (cm)	Radicle length (cm)
Control	97±1.53 <sup>c</sup>	2.64 ± 0.16 <sup>c</sup>	18.17 ± 0.19 <sup>c</sup>
BLC	92±2.00 <sup>a</sup>	1.66 ± 0.13 <sup>c</sup>	9.83 ± 0.87 <sup>a</sup>
OELC	95±2.24 <sup>a</sup>	1.28 ± 0.16 <sup>d</sup>	2.50 ± 0.11 <sup>c</sup>
OMLC	92±2.50 <sup>a</sup>	1.98 ± 0.06 <sup>b</sup>	10.85 ± 0.32 <sup>a</sup>
TMLC	86±4.27 <sup>b</sup>	2.32 ± 0.08 <sup>a</sup>	6.43 ± 0.10 <sup>b</sup>

BLC = Below *Lantana camara*, OELC = On the edge of *Lantana camara*, OMLC = One meter away from *Lantana camara* TMLC = Two meter away from *Lantana camara*

### 4.2.2. Effect of soil bed

Similar to soil extract treatment, percent of seed germination was significantly reduced when seeds were sown on soil bed sampled from two meters away from lantana thicket. However, there was no significant difference between other soil beds. Plumule length was significantly higher for seeds sown on soil bed sampled from two meters away from lantana thicket, whereas radicle length was higher for seeds sown on soil bed sampled from one meter away from lantana canopy (Table 3).

The variations observed between treatments may be ascribed to the differential distribution of some compounds/hormones that may stimulate or inhibit germination, and plumule and radicle growth.

**Table 3. Germination parameters of seeds of *Brassica carinata* sown on soil bed of soil samples from underneath *Lantana camara* canopy and away from *Lantana camara* in distance gradient.**

Treatments	Germination rate (%)	Plumule length (cm)	Radicle length (cm)
Control	90±2.11 <sup>ab</sup>	2.85±0.14 <sup>a</sup>	9.5±0.26 <sup>b</sup>
BLC	92±2.00 <sup>a</sup>	1.66 ± 0.13 <sup>c</sup>	9.83 ± 0.87 <sup>a</sup>
OELC	95±2.24 <sup>a</sup>	1.97 ± 0.06 <sup>b</sup>	6.76 ± 0.21 <sup>b</sup>
OMLC	92±2.50 <sup>a</sup>	1.98 ± 0.06 <sup>b</sup>	10.85 ± 0.32 <sup>a</sup>
TMLC	86±4.27 <sup>b</sup>	2.32 ± 0.08 <sup>a</sup>	6.43 ± 0.10 <sup>b</sup>

BLC = Below *Lantana camara*, OELC = On the edge of *Lantana camara*, OMLC = One meter away from *Lantana camara*, TMLC = Two meter away from *Lantana camara*,

#### **4.3 Germination and growth performance of *B. carinata* grown on soil sampled from different sites in distance gradient from *L. Camara* under greenhouse**

There was no significant difference in days to complete germination between seeds grown on soil samples obtained in distance gradient from underneath lantana canopy. However, germination was completed earlier on soil from the edge followed by from two and one meters away from lantana canopy. Seeds sown on soils from beneath lantana canopy completed germination late (Table 4). Delayed germination of test species under allelopathic stress might be due to imposition of dormancy on seeds by allelochemicals and seeds might germinate whenever conditions turn favorable due to loss of allelopathic substances from soil (Nasr and Shariati, 2005).

In greenhouse experiment significant difference was observed between soils of different distance gradient from *B. carinata* in seed yield. This may be due to the effect of allelochemicals released by allelopathic donor plants depend upon a number of factors including accumulation capability and microbial activity. Previously, Achhireddy and Singh (1984) indicated that soil collected from under *Lantana* had no effect on germination and growth of milkweed vine.

Contrary to this, Senarathne *et al.* (2011) demonstrated germination inhibitory effect of *Lantana* invaded soils on seed germination of *Lycopersicum esculentem* and *Capsicum annum*.

**Table 4. Impact of soils from different distance gradient on *B. carinata* seed germination and seed yield**

Treatments	Days to complete germination	Percent germination	Seed weight (gm)
Control	5.00±00 <sup>b</sup>	81.7±2.18 <sup>a</sup>	335.3±1.78 <sup>a</sup>
BLC	6.00 ± 0.21 <sup>a</sup>	74.30 ± 2.89 <sup>ab</sup>	144.10 ± 1.57 <sup>c</sup>
OELC	5.60 ± 0.22 <sup>a</sup>	71.00 ± 3.14 <sup>b</sup>	178.40 ± 1.69 <sup>a</sup>
OMLC	5.80 ± 0.20 <sup>a</sup>	77.00 ± 3.00 <sup>ab</sup>	172.10 ± 0.84 <sup>b</sup>
TMLC	5.70 ± 0.21 <sup>a</sup>	71.00 ± 4.82 <sup>b</sup>	77.00 ± 1.26 <sup>d</sup>

BLC = Below *Lantana camara*, OELC = On the edge of *Lantana camara*, OMLC = One meter away *Lantana camara*, TMLC = Two meter away *Lantanacamara*,

#### **4.4. Some soil chemical proerties measured from soil sampled from different sites relative to beneath *Lantana* canopy**

Soil pH was found to decrease with increasing distance from beneath *Lantana* canopy. Available phosphorus and total nitrogen were relatively higher in soils collected from 1 and 2 m away from *lantana* canopy, while organic matter is relatively higher at one meter away from *Lantana camara* canopy (Table 5). The present investigation corroborate with Ruwanza and Shackleton (2016) which indicated that the soils from underneath *Lantana camara* had a lower level of total P compared to the soils in the adjacent sites, while the OM showed no significant difference between the invaded and un-invaded sites.

**Table 5. Some chemical properties of soils sampled at distance gradient from beneath *Lantana* canopy**

Soil sample	pH	Total N %	Available P (ppm)	%OM
Control	7.31	0.22	7.88	1.68
BLC	7.75	0.42	18.38	4.40
OELC	7.55	0.48	23.85	4.45
OMLC	7.24	0.56	27.88	4.64
TMLC	7.05	0.48	30.17	4.00

BLC = Below *Lantana camara*, OELC = On the edge of *Lantana camara*, OMLC = One meter away from *Lantana camara*, TMLC = Two meter away from *Lantana camara*

## 5. SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

### 5.1 Summary and Conclusions

*Lantana camara* is one of the most invasive plant species which is widely distributed worldwide. It produces and releases several secondary metabolites (allelochemicals) such as phenolic acids, flavonoids, terpenes, quinines and essential oils, and *etc.* Moreover, under field conditions the presence of this weed is one of the major factors responsible for growth and yield reduction in crops. The findings of this research work revealed that allelopathic interactions between crops and weeds were also partly responsible for such losses in crop yields. *Lantana camara* is one of the most invasive weeds which have allelopathic effect on other plants. But, little information is available on phytotoxicity effect of *Lantana camara*. On the other hand, in Ethiopia such as Oromia, Babile and Somali regions most of the lands are occupied by the weed *Lantana camara*.

Therefore, in this study an investigation was carried out under laboratory and greenhouse conditions at biology laboratory and *Rarre* green house, respectively with the objective of evaluating the allelopathic effects of leaf extracts of *Lantana camara* and growth inhibitory effect of soil invaded by it on *Brassica carinata*. Laboratory bioassay on germination and seedling growth of the test crop was investigated on water, methanol and hexane leaf extracts.

The results showed that solvents (water, hexane and methanol) extracts of *Lantana camara* have allelopathic effect of *Brassica carinata*. Germination parameters and growth performance of the test plant were inhibited depending on type of solvents. Comparison between water, hexane and methanol extracts showed that water extracts had higher effect on the measured parameter than hexane and methanol extract. However, soil residual toxicity test made in the laboratory and greenhouse, the inhibitory effect depended on the distance of the soil collected from *lantana* site. In greenhouse experiment significant difference was observed between soils of different distance gradient from *Lantana camara* in seed yield of *Brassica carinata*.

## **5.2 Recommendations**

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

- ✓ Since the study considers only allelopathic potential of *Lantana camara* on Germination and growth performance of *Brassica carinata* in greenhouse condition for one season, it is recommended to have additional investigations in field condition.

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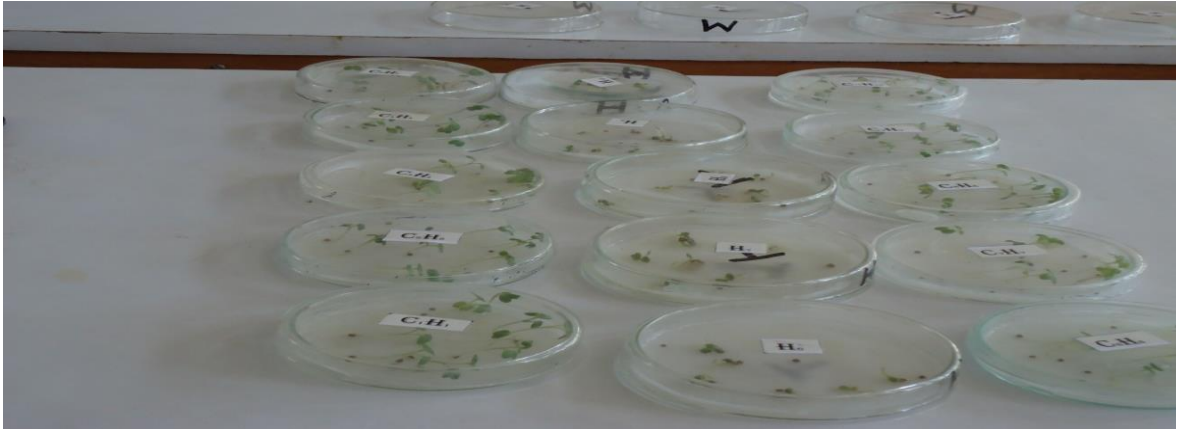
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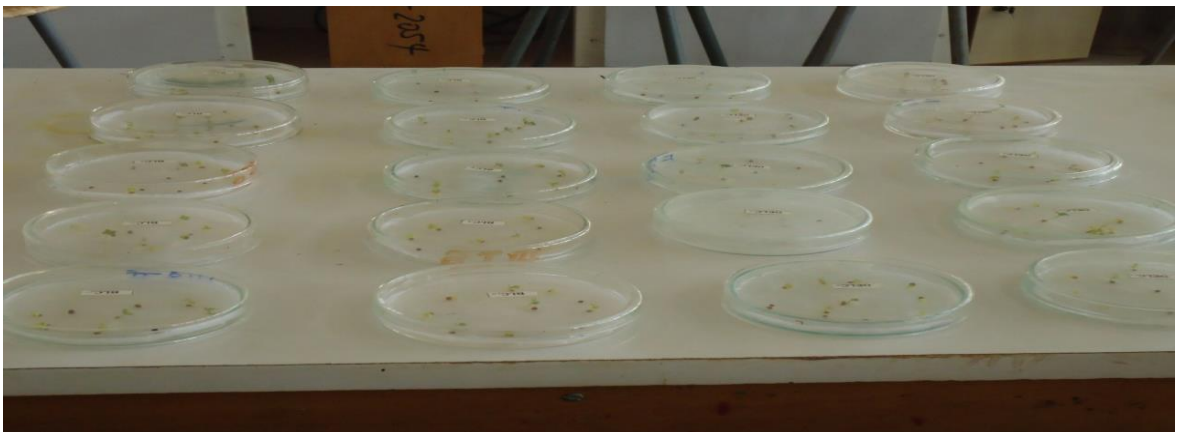
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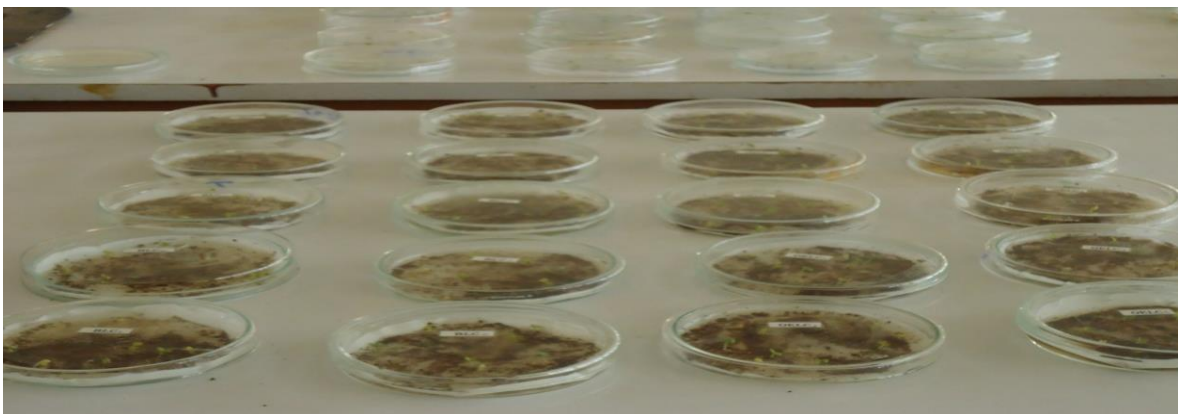
## 7. APPENDIX FIGURES



Appendix 1. Brassica seed germinated in laboratory with water, hexane and methanol extract



Appendix 2. Brassica seed germinated in laboratory on *lantana* soil extract (soil extract bioassay)



Appendix 3. Brassica seed germinated in laboratory on lantana infested soil (soil bed bioassay)



Appendix 4. Brassica seed grown in green house on different lantana infested soil



Appendix 5. Brassica seed grown on non-lantana soil (control)



## Appendix 6. Brassica plant reaches maturity