

**EVALUATION OF BOTANICAL HERBICIDES AGAINST COMMON
WEED SPECIES OF COFFEE (*Coffea arabica* L.) WITH EMPHASIS
ON *BIDENS PILOSA* AT JIMMA, SOUTHWESTERN ETHIOPIA**

MSc THESIS

ABERA DABA BIRR

DECEMBER 2012

JIMMA UNIVERSITY

**EVALUATION OF BOTANICAL HERBICIDES AGAINST COMMON
WEED SPECIES OF COFFEE (*Coffea arabica* L.) WITH EMPHASIS
ON *BIDENS PILOSA* AT JIMMA, SOUTHWESTERN ETHIOPIA**

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**In Partial Fulfillment of the Requirements for the Degree of Master of
Science in Horticulture (Coffee, Tea and Spices Science)**

Abera Daba Birr

December 2012

Jimma University

APPROVAL SHEET
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As thesis research advisors, we hereby certify that we have read and evaluated this thesis prepared, under our guidance, by **Abera Daba Birr**, entitled **Evaluation of Botanical herbicides against Common Weed Species of Coffee (*Coffea arabica* L.) with emphasis on *Bidens pilosa* at Jimma, Southwestern Ethiopia**. We recommend that it be submitted as fulfilling the thesis requirement.

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DEDICATION

I dedicate this manuscript to my brother Bekele Daba who planted the seed of astuteness within me, from which my thirst for knowledge grew. Also I dedicate this work to my advisor Mr. Mulatu Wakjira who played vital role for this work. It was during the thesis work I have been regrettably disturbed by the death of Mr. Mulatu Wakjira who did not last to see the fruit of this work.

STATEMENT OF THE AUTHOR

First, I declare that this thesis is my own work and that all sources of materials used for this thesis have been dully acknowledged. This thesis has been submitted in partial fulfillment of the requirements for MSc degree at the Jimma University and to be made available at the University's Library under the rules of the Library. I seriously declare that this thesis is not submitted to any other institutions anywhere for award of any academic degree, diploma, or certificate.

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BIOGRAPHICAL SKETCH

Abera Daba Birr, the author, was born on September 20, 1984 at Oromia Region, Western Shewa zone, Jaldu Woreda. He attended his elementary school at Jaldu Elementary and Junior Secondary School. He then pursued his high school education in Ambo at Ambo Comprehensive Secondary School. Following the completion of his high school education, he joined Jimma University College of Agriculture and Veterinary Medicine and graduated with BSc Degree in Horticulture in July, 2006. After graduation he was employed at Tepi Coffee Plantation Development Enterprise where he had served for one year as super visor. Then he was employed at Mizan Agriculture Technical, Vocational and Educational Training College and served for a year as junior instructor. During his stayed in the college, he had offered different courses for trainees under the department of plant sciences. Lastly, he was employed by Mizan-Tepi University where he had served for a year as an assistant lecturer and head department of Horticulture until he joined the School of Graduate Studies of Jimma University College of Agriculture and Veterinary Medicine to pursue a graduate study leading to a Master of Sciences Degree in Horticulture (Coffee, Tea and Spices Science).

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ABBREVIATIONS

BPEDORS	Bureau of Planning and Economic Development of Oromia Regional State
cm	centimeter
DPI	Department of Primary Industries
EAFCA	Eastern African Fine Coffees Association
ECFF	Ethiopian Coffee Forest Forum
EIAR	Ethiopia Institute of Agricultural Research
EOF	Ethiopian Organic Forum
FAO	Food and Agriculture Organization
G/ER	Germination/Emergence Rate
g	Gram
ha	Hectare
H ₂ O ₂	Hydrogen per oxide
ICO	International Coffee Organization
IFOAM	International Federation of Organic Agriculture Movements
ITC	International Trade Centre
JARC	Jimma Agricultural Research Center
JUCAVM	Jimma University College of Agriculture and Veterinary Medicine
ml	Milliliter
NCFAP	National Center for Food and Agriculture Policy
PANE	Pesticide Action Network Europe
PIER	Pacific Island Ecosystems at Risk
v/v	volume by volume
WHO	World Health Organization

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EVALUATION OF BOTANICAL HERBICIDES AGAINST COMMON WEED SPECIES OF COFFEE (*Coffea arabica* L.) WITH EMPHASIS ON *BIDENS PILOSA* AT JIMMA, SOUTHWESTERN ETHIOPIA

ABSTRACT

Despite the wealth of genetic diversity, the yield of Ethiopian coffee is low compared with other producer countries. Weeds are one of the most limiting constraints of crop production particularly in organic farming systems, as no herbicides are allowed due to the intent of curtailing their negative impacts on the environments, health and sustainability reasons. Similarly, techniques like mechanical, cultural, biological etc. can be costly and may fail to control weeds adequately. Using natural products, like bioherbicides, are among possible alternatives for weed control in organic farms. Therefore, a series of laboratory, lath-house and field experiments were conducted in 2011/12 at Jimma, Southwestern Ethiopia with the objective of exploring botanical herbicides against common weeds of coffee with emphasis on *B. pilosa*. The botanical extracts evaluated in the present study consisted of *Artemisia annua*, *Rosmarinus officinalis*, *Trachyspermum ammi*, *Cymbopogon winterianus*, *Eucalyptus citrodora*, *Eucalyptus globulus*, *Piper nigrum*, *Ricinus communis* and inert minerals (Diatomate, Bole, and H_2O_2) combined with concentrations (0.03, 0.06 and 0.09% (v/v)) in CRD for laboratory, application frequency (1x, 2x and 3x) in RCBD with 5% (v/v) concentration for lath-house and field experiments. For comparison, a control and standard check were included and a factorial arrangement was used for all experiments with three replications. In view of that, data were collected on germination, growth and growth parameters of *B. pilosa* seeds and common weeds of coffee. The results under laboratory revealed that the differences among tested materials were very highly significant ($P < 0.001$) for all parameters studied. The least germination per cent (2%) was obtained as a result of seeds treated with *E. citrodora* and *C. winterianus* at 0.09% (v/v) compared to control (98%). Germination rate was fastest for seeds treated with only distilled water (54.42 seeds day⁻¹) and the lowest was recorded from *C. winterianus* (0.16 seeds day⁻¹) at 0.09% (v/v). Shoot and root length of *B. pilosa* were very highly significant ($P < 0.001$) variation. The inhibitory effect of tested materials under lathhouse was not as effective as laboratory experiments. Regardless of these differences, oils extracted from *C. winterianus*, *E. citrodora*, *R. officinalis* and *E. globulus* revealed consistencies inhibitory effects at both conditions. Essential oils from *E. citrodora*, *C. winterianus* and *T. ammi* also checked their potential herbicides against common weeds of coffee at field trial and the result revealed that highly significant for first two botanicals and no difference for *T. ammi* related to control for all growth parameters. From these findings, essential oils from *E. citrodora* and *C. winterianus* revealed consistent inhibitory effects on growth and growth parameters of weed species at various experimental conditions. Therefore, organic coffee farming systems in Ethiopia may use these oils as alternative means of weed control even though further study needs to be conducted to know the active ingredients inherited from the source plants, mode of action, rate as well as time of application to come up with practical recommendation.

1. INTRODUCTION

Agriculture remains the key sector for the economic development for most developing countries. It is critically important for ensuring food security, alleviating poverty and conserving the vital natural resources that the world's present and future generations will be entirely dependent upon for their survival and well-being (Ananata, 2002). Coffee has become a major global commodity and the topmost valuable primary products in world trade (Martin *et al.*, 2002). After oil, coffee is the second most valuable traded commodity worldwide, with global retail sales estimated to be US\$ 90 billion and it is the major export product of some countries such as Uganda, Burundi, Rwanda and Ethiopia (DaMatta *et al.*, 2007). The world annual coffee production is around 7 million tons, of which Brazil produces one-third. Several countries in Africa base their economies on coffee export revenues (Aklilu and Eva, 2010). Africa produces about 10% of total world coffee. In production of Arabica coffee, Ethiopia is the number one producer in Africa and the sixth largest producer in the world, turning out 3.5% of the world coffee in the 2008/09 (EAFCA, 2010; Abu, 2012).

Ethiopia is the country where coffee was first discovered and spread to the other world (Tora, 2009). More genetically diverse strains of *C. arabica* exist in Ethiopia than anywhere else in the world, which has lead botanists and scientists to agree that Ethiopia is the centre for origin, diversification and dissemination of the coffee plant (Bayetta, 2001). Dominic *et al.* (2011) and Abu (2012) reported that, coffee is Ethiopia's number one source of foreign exchange earning accounting for more than 35%. According to these authors and ITC (2011) the production of coffee is mainly in West and South Ethiopia, around 40-50% based on garden coffee production system.

Although Ethiopia is the home of *C. arabica* with high genetic diversity, paradoxically, the national average yield is between 0.34 and 0.49 ton per ha of clean beans, which is lower as compared with other coffee producing countries, for instance, in Brazil (1.4-1.6 tons per ha) or Colombia (above 1.5 tons per ha) (Aklilu and Eva, 2010). The main reasons for the low yield were poor management, limited supply of improved coffee cultivars, diseases and insect pest problem, and the lack of initiative to produce more owing to the low and fluctuating coffee price (Girma *et al.*, 2008).

Weeds compete with coffee for moisture and plant nutrients, while some perennial grasses and sedges produce root exudates that are toxic to coffee. Weed control is particularly difficult in unshaded coffee (Van Der Vossen, 2005). Most farmers in Ethiopia do not weed their fields at the right time because of labour bottlenecks. Research by the Institute of Agricultural Research throughout the country studied the effects of delayed weeding on crop yields (Kebede, 2000). According to the finding, the yield reduction in coffee as a result of delayed weeding is about 62%. As a result of weed competition, coffee yield and quality are seriously decreased and weed management is one of the major operation and all year round agricultural activities which entails high cost in coffee production (Ronchi and Silva, 2006). Average cost (per ha/year) for weed control in organic farming system in Ethiopia is US\$25 (Rani *et al.*, 2007).

Table 1: Some common weeds found in coffee growing at the study area

Common names	Botanical names	Growth habit	Status
Black Jack	<i>Bidens Pilosa</i> L.	Annual broad leaved weed	Major
Pigweed	<i>Amaranthus</i> spp.	>>	>>
Goat weed	<i>Ageratum conyzoides</i> L.	>>	>>
Gallant Soldier	<i>Galinsoga parviflora</i>	>>	>>
Star Grass	<i>Cynodon nelmefensis</i> (Venderyst)	Perennial grasses	>>
Couch grass	<i>Digitaria scalarum</i>	>>	>>
Blue coach grass	<i>Digitaria abyssinica</i> (A. Rich) stapf	>>	>>
Yellow nut sedge	<i>Cyperus esculentus</i> L.	Perennial sedge	>>
Purple nut sedge	<i>Cyperus rotundus</i> L.	>>	>>

Source: Girma *et al.* (2008)

Conventional farming has been largely dependent on intensive inputs of synthetic fertilizers and pesticides. The systems are often associated with problems such as disturbances of the environment, groundwater pollution and lethal effect to non target organisms in the agro-ecosystems in addition to direct toxicity to users (Poudel *et al.*, 2002; Anand *et al.*, 2008). Beside, recently, there has been considerable media attention to the potential development of “super weeds” that would be resistant to all herbicides (Matt *et al.*, 2001; NCFAP, 2003). According to the report by GM Freezez, (2011) Weed resistance is now a significant agronomic, socio-economic, health and environmental problem in areas where Roundup Ready genetically modified herbicide tolerant crops have been grown over a number of years and where Roundup/glyphosate has been the only, or

very dominant, means to control weeds. 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 (Taye, 2007). Due to these and other sustainability reasons, many countries are attempting to reduce the reliance on synthetic herbicides for weed management and led to the development and promotion of organic farming system that account of the environment and public health as main concerns (Araujo *et al.*, 2008).

Organic farming seems to be more appropriate, it considers the important aspects like sustainability of natural resources and environment. It is a production system which favors maximum use of organic materials and discourages use of synthetically produced agro-inputs (Ananata, 2002). On the other hand, routinely reports that weed control without chemicals is their biggest problem. Organic growers use a variety of nonchemical techniques for weed control including cover crops, rotations, flammers, vinegar, tillage, hand weeding and plastic sheets for smothering weeds which provide partial weeds control (NCFAP, 2003). These techniques are intensive and slow compared to other methods like chemicals, and may damage crop roots (Kebede, 2000). Therefore, it has now become necessary to search for the alternative use of bio-herbicides is considered potential means of pest control, which can minimize the use of synthetic pesticides.

Natural product-based pesticides are generally considered safer than synthetic pesticides because of their relatively short environmental half-life. One of the indirect and important benefits of the chemical composition and structural characteristics of natural products (*e.g.*, the absence of ‘unnatural’ ring structures and the low amount of heavy atoms) of these compounds are rapidly degraded in the natural environment (Franck *et al.*, 1998). The botanical insecticides are generally pest-specific and are relatively harmless to non-target organisms including man (Adeyemi, 2010).

There are currently several herbicides being marketed as “organic” for home garden use. All are derived from plant oils and work by inhibiting plants’ respiration causing rapid desiccation and brown-off. Organic herbicides must be derived from natural substances by physical means and not be chemically transformed in any synthetic way. They are not allowed to be combined with any synthetic substance before use (James and Rahman, 2005). Naidu and Raghuramulu (2000) also said that a number of naturally derived

products have been approved for disease and pest control in organic coffee production, including Bordeaux mixture, neem oil and leaf extracts, nicotine, rotenone and pyrethrum.

Synthetic pesticides have drawbacks as stated by many authors and it is time to search alternative pesticides which have less impact on environment, and pose a little risk to human health. According to Buss and Park-Brown (2009) alternatives include natural products, such as horticultural oils, mineral and botanical pesticides. However, at present these alternatives are not comparable to synthetic chemicals because they are not readily available on the market, at least not in Ethiopia, and their efficacy in comparison with synthetic products might be questioned. But there is a promising future for these natural products because they are becoming increasingly important (Haiké and Sue, 2007).

The development of natural product-based herbicides is especially relevant for coffee weed management by small-scale farmers in developing countries like Ethiopia, where coffee is produced as garden coffee by small-scale farmers. Moreover, plant essential oils can be used as a component of integrated weed management of coffee weeds, which is the best option for small-scale farmers (Isman, 2008). Hence, the development of organic herbicides from locally available plant sources for weed management package particularly with regard to optimization of organic coffee production is imperative.

1.1 Hypothesis

In order to investigate the potential of essential oils from local plants and some activated naturally occurring inert minerals for management of weeds in organic coffee in Ethiopia, the following hypotheses were formulated: (1) different essential oils and activated naturally occurring inert minerals might have different weed control potentials, (2) there might be variations in inhibitory effects due to variation in application concentration and frequency of tested products and (3) laboratory bioassay may give substantial clues about the expected results from lathhouse and field experiments conditions.

1.2 Objectives and Experimental Approach

This thesis research was initiated in order to evaluate the efficacy of botanicals (plant extracts and activated naturally occurring inert minerals) for ecological management of weeds in organic coffee system at Jimma, Southwestern Ethiopia. The specific objectives were: (1) to examine the efficacy and determine optimal concentration of different botanical herbicides for inhibition of *B. pilosa* seeds germination and early growth under laboratory conditions (2) to evaluate the efficacy of application frequency of different botanical herbicides on *B. pilosa* plant under lathhouse conditions (3) to evaluate the efficacy of application frequency of different botanical herbicides on common weeds of coffee at field conditions. To meet these objectives, laboratory, lathhouse and field experiments were conducted under three conditions. The first experiment attended to detect herbicidal effects of different plant extracts and some locally collected inert minerals with different concentration on *B. pilosa* seed germination under laboratory condition in Petri dish. The second experiment addressed the inhibitory potential of essential oils extracted from local plants and some inert minerals on growth of *B. pilosa* in Pot-culture under lathhouse conditions. The third experiment (field trial) determined potential herbicidal activity of candidate oils from botanicals that were identified in the lathhouse screening. Finally, future research needs regarding bioherbicide-mediated weed management for organic coffee production systems were addressed.

2. LITERATURE REVIEW

2.1 A Brief Overview of Coffee

Coffee is a non alcoholic stimulant beverage crop plant (Bayetta, 2001) and belongs to the genus *Coffea*, in the Rubiaceae family. There are about 103 species of genus *Coffea*, all exclusively restricted to the tropical forests of Africa, Madagascar and islands of the Indian Ocean (Mascarene Islands). Of all the species, only two (*Coffea arabica* L.) and (*Coffea canephora* Pierre) have commercial value in the world coffee industry (Taye, 2010). From the genus *Coffea*, arabica is the only tetraploid and self-fertile species with chromosome number of $2n = 4x = 44$. The coffee plant is a woody perennial evergreen small tree that can grow up to 15 m height. The tree has a central main stem called orthotropic and primary, secondary, and tertiary and even more horizontal branches known as plagiotropic. The coffee plant needs two to four years from field planting to first harvest (Charrier and Eskes, 2004). *Coffea arabica* is the only species occurring in Ethiopia and is geographically isolated from the rest of the *Coffea* species and the most popular and widely cultivated in the world, dominating 70% of total coffee production and over 90% of the market (Taye, 2010).

2.2 Economic Importance of Coffee

Coffee is the single most important tropical commodity traded worldwide, accounting for nearly half of total exports of tropical products (FAO, 2009). Coffee has become a major global commodity and the topmost valuable primary products in world trade (Martin *et al.*, 2002). Worldwide it is estimated that there are 25 million producers of coffee, with the main centers in Latin America, Africa and Asian counties. For many of these countries, coffee production was seen as an obvious revenue generator (Woods, 2003). According to the report ICO (2009) over 50 countries produce coffee in significant amounts; in many of these, foreign exchange earnings from coffee exports are of vital importance to the balance of payments. Coffee is an important agent of development, generating cash returns in subsistence economies and, since coffee production and harvesting are labour-intensive, providing an important source of rural employment, for both men and women.

Several countries in Africa base their economies on coffee export revenues (Aklilu and Eva, 2010) and Ethiopia, like other countries became highly dependent on this commodity hence earnings foreign exchange for the country and the cultivation of this crop plays a vital role both in the cultural and socio-economic life of the nation (Woods, 2003; ECFE and Robera, 2007). The coffee sub-sector is also important in terms of providing income for a large number of households and provides jobs for many more people in coffee-related activities. About 25% (almost 20 percent of the total population) of the Ethiopian population depend, directly or indirectly, on coffee production, processing and marketing (ECFE and Robera, 2007; Samuel and Eva, 2008; Abu, 2012).

2.3 Organic Coffee Production

Organic Agriculture should sustain and enhance the health of soil, plant, animal, human and planet as one and indivisible. This principle points out that the health of individuals and communities cannot be separated from the health of ecosystems-healthy soils produce healthy crops that foster the health of animals and people (IFOAM, 2009). The important economical impacts may be organic farming will be 40.6% cheaper than chemical farming and hence income security in peasant, reduction of cash investment, more return, low risk and self-sufficient and stronger rural economy (Rani *et al.*, 2007). Organic farming is gaining worldwide acceptance and has been expanding annually in the last decade, accounting for over 24 million hectares worldwide (Araujo *et al.*, 2008).

Organic farming is the way towards sustainable development for a developing country like Ethiopia (Rani *et al.*, 2007). Organic agriculture is, by its nature, more labour intensive than industrial agriculture and its concepts also encourage sustainable use of domestic raw materials. Therefore, it makes sense for Ethiopia to also compete globally in organic agriculture sector where the market has been growing annually by more than 25%. Besides, the current global concern on climate change, green house gas emission, degradation of the natural resource base and dramatic decline in soil productivity has created a favorable condition for organic agriculture to get focus in the country (EOF, 2009).

Organic coffee is grown as part of an intensive, holistic agricultural production management system that includes the composting of organic materials, mulching, shade regulation and biological pest control. Such a system is based on the principle that a value corresponding to that harvested should be returned to the soil (ITC, 2002). Organic coffee cultivation is of economic importance mainly in Mexico, Peru, Guatemala, Bolivian and in the Dominican Republic. Until now, organic cultivation has been of less importance in such regions as Costa Rica, Brazil, Columbia, Venezuela, Indonesia, India, Ethiopia, Kenya and Mozambique (Naturland, 2000). The status of research progress in the organic coffee sector in Ethiopia, is at its infancy, and it is difficult to find a holistic technological innovation which is able to provide for farmer's immediate solutions to basic problems (Mekuria *et al.*, 2004).

In Ethiopia, onset of certified organic farming emerged from a market deficit of the conventional coffee farming, but there is little information documented about such important segment of coffee production and the country's economy. According to Mekuria *et al.* (2004) about 95% of coffee produced in Ethiopia is *de facto* organic. However, certified organic coffee production accounts for only an estimated 0.1% of the total national coffee production in Ethiopia. The increasing growth of the organic Arabica coffee sector in Ethiopia might contribute to a better economic situation of small-scale farmers and the conservation of the genetic diversity of high quality Arabica coffee land races.

Globally, organic agriculture has many challenges and, at the same time, a lot of opportunities. These are true also for the development of the organic sector in Ethiopia. According to Mekuria *et al.* (2004) there are enormous potentials for developing of Organic Agriculture in Ethiopia, with coffee farmers in a position to pioneer certified organic crop production. The main challenges of the organic sector development in Ethiopia are inadequate knowledge of organic techniques by farmers, exporters, processors, lack of Governmental and/or private extension service and training, lack of low cost of certification techniques, lack of high quality and consistency organic product supply, not well developed local market and specialized research and weak network in coordination and mobilization of human and financial resources (EOF, 2009).

The inherent growth of coffee is affected by several factors, including drought, temperature, photoperiod, water logging and leaching of nitrates by high rainfall. The major climatic factors include temperature, water, light and wind (Wrigley, 1988). Climatic variability has always been the main factor responsible for the fluctuation of coffee yields in the world, *climate change*, as a result of global warming, is expected to result in actual shifts on where and how coffee may be produced in future. This will affect millions of producers as well as all other participants in the value chain, right up to the end-consumer and presents a major challenge to the coffee industry (Alexander, 2010). There is a close relationship between soil moisture and stomatal opening in this plant. When soil moisture becomes high, stomata open which is a common behavior of other plants too. Hence, water besides its role in nutrient absorption, translocation and as raw material for photosynthesis can affect plant like other plants by its influence indirectly on the opening and closing of stomata. This finally affects the rate of photosynthesis, CO₂ assimilation and in turn yields (kassahun, 2001).

Diseases and insect pests are another challenge in coffee sub-sector and the major coffee diseases in Ethiopia are coffee berry disease (CBD), coffee wilt disease (CWD) and coffee leaf rust (CLR). The most important diseases both in severity and wide distribution are coffee berry disease (CBD) and coffee wilt disease (CWD) (Arega, 2006). According to the author, coffee insect pests, mainly antestia, leaf miners and coffee berry borer, cause damage in most coffee growing regions of the country. Weeds are recognized as one of the main bottlenecks in organic farming in general and organic coffee production in particular.

2.4 Weeds: One of the Bottlenecks of Coffee Production

A plant is considered a weed if it has certain characteristics that set it apart from other plant species (Pennstate, 2007) and interfere with emergence, growth and yield of crops through competition and allelopathy mechanisms. Weeds compete with crops for moisture, nutrients, sunlight and space thereby resulting in significant crop losses (NCFAP, 2003). Other problems associated with weeds include reducing crop quality by contaminating the commodity, interfering with harvest, serving as hosts for crop diseases or providing shelter for insects to overwinter, limiting the choice of crop rotation sequences and cultural practices, producing chemical substances that can be allergies or toxins to humans, animals, or crop plants (allelopathy) (Pennstate, 2007). Allelopathy is a

mechanism in which chemicals produced by weed plants may increase or decrease the associated plant growth. The plant may exhibit inhibitory or rarely stimulatory effects on germination and growth of other plants in the immediate vicinity (Jabeen and Ahmed, 2009).

A range of weeds are associated with coffee, ranging from annual grasses and broad leaved weeds, which are relatively easy to control, to persistent perennials that are more difficult to manage. Perennial grass species reproduce through underground stolons, tubers and rhizomes, such as couch grasses. A number of broad leaved species such as *Oxalis* spp. and *Parthenium* spp. are also important in coffee, although most species are less harmful than perennial grasses (Martin *et al.*, 2002). In a previous survey, a total of over 63 weed species from 23 families were identified in Ethiopia (Getachew, 1991).

As a result of weed competition, coffee yield and quality are reduced as coffee feeder roots tend to lie close to the surface whereas competitive weeds send their roots deep to tap the underlying moisture, hence, weed control is one of the major cultural operations that require high cost and time (Girma *et al.*, 2008). High rainfall and hot humid climate encourage rapid and continuous growth of the noxious perennials, such as *Cyperus* spp., *Cynodon* spp, and *Digitaria* spp that seriously compete with crops and result in enormous yield reduction. For instance, yield loss assessment studies conducted in Jimma showed a yield loss of 60-80% for coffee (Tadesse *et al.*, 2007). On the other hand, total N content of coffee seedlings was reduced by 49 percent when couch grass was allowed to compete full season (Tadesse, 1994 cited in Girma *et al.*, 2008). These results clearly indicate that weeds can seriously compete with coffee bushes and weeding is a vital operation in coffee production. Weeds differ from most pests and diseases in that killing or removing them by direct physical means is a practical option. The problem is in removing the weeds selectively without injuring the crop. On small areas or where sufficient work force is available hand-weeding remains a possibility, particularly in high value crops, but on most farms, crops are grown on too large a scale, and labour is expensive and often of limited availability (Bond *et al.*, 2003).

Bidens pilosa L. is a species in the plant family Asteraceae. It is a small, erect annual herb that grows to 1 m high. It has bright green leaves with serrated, prickly edges and produces small, yellow flowers and black fruit (Adeolu *et al.*, 2011). It is troublesome

weed to at least 30 crops in over 40 countries and it is known to significantly reduce crop yields. It is an important weed of pastures, maize, sorghum, vegetables, cotton, tea, coffee, cassava, coconut, oil palm, citrus, papaya, rice, rubber and tobacco. *B. pilosa* prevents the regeneration of these plants as well, given its allelopathic properties. Leaf and root extracts are known to significantly suppress germination and seedling growth of many plants and are believed to remain active throughout decomposition. Furthermore, *B. pilosa* grows three times faster than similar plant species. This weed also a host and vector to harmful parasites such as root knot nematodes (*Meloidogyne spp.*) and Tomato spotted wilt virus (*Sclerotinia sclerotiorum*) (Mvere, 2004; DPI, 2008). A single plant may produce 3,000 to 6,000 seeds per year which are spread by attaching to animals, birds, and people or dispersal by wind and water. Seeds are reported to have no dormancy, remain viable for five to six years, and a 74% germination rate in the field (PIER, 2007; DPI, 2008). *B. pilosa* grows quickly and plants flower four months after germination and produce mature seeds four weeks after flowering. Plants typically bear 80 flower heads with seeds with potential production of 3000 plants in a generation and four generations per year (Mvere, 2004; PIER, 2007; DPI, 2008).

2.5 Weed Management

Pest control, among other agronomic activities, should be improved by minimizing the use of chemical pesticides through the rational and judicious use of different control methods that are technically effective, economically feasible to smallholders and environmentally safe. Weed management still relies mainly on the use of herbicides in developed economies while hand-weeding is the main control method in the developing world (Labrada, 1997). Weed management is one of the main concerns in organic agriculture. Generally, all aspects of arable crop production play an important role in a system approach to problems. The elements to consider in preventing weed problems are crop rotation, mulching and tillage may also be useful in weed control (Ananata, 2002).

Weed control is one of the most difficult problems in organic farming where herbicides are prohibited. This is one of the major reasons for large-scale conventional coffee growers not to convert to organic production (Beveridge and Naylor, 1999). Mechanical weed control can offer a partial solution to the weed problem. Slashing and digging are the major methods of weed control employed by majority of both organic and conventional

coffee growers (Kassahun, 1994). However, digging can harm the shallow root system of the coffee plant and need labor and time, while; slashing can transmit coffee wilt disease caused by *Gibberella xylarioides*, one of the most important fungal diseases of coffee (Girma *et al.*, 2005).

A number of weed control strategies are available to smallholders. Cultural and mechanical controls include weed slashing, using a machete, and the use of a mulch or cover crop which can be effective for many grass weeds such as couch and star grass. Hoeing may be used in some systems, but is not recommended where coffee is grown on slopes, as it may increase soil erosion (Martin *et al.*, 2002). Since weeds are so prevalent in many areas of the landscape, management techniques are necessary to maintain order. Weed management is most successful when it involves an integrated approach using a variety of methods. The common methods used to manage weeds include prevention and cultural, mechanical, biological, and chemical means.

2.5.1 Cultural/ecological weed management

Cultural control practices make the environment less favorable to weeds that include exclusion, crop rotations, seedbed preparation and cover crops. To be successful, cultural controls require skillful management. They also require growers to consider prevention as well as control, and to optimally combine practices to achieve the best results (Final IPM Plan, 1998). Philip *et al.* (2002) stated that cultural weed control includes those management practices that modify the agroecosystem to make the pasture, crop, or forest ecosystem resistant to weed establishment and, at the same time, support overall economic goals .

Crop competition can be an inexpensive and effective aid to weed management if used to its fullest advantage. Examples of cultural techniques include following soil test recommendations for fertilizer and lime; selecting the best crop varieties; planting dense crop populations at the proper timing; scouting fields regularly for weeds, insects, and diseases and controlling them when necessary; and including crop rotations in the system. Composting, ensiling, or feeding weeds or weed-infested crops to livestock can destroy the viability of weed seeds. The heat and/or digestive acids break down the majority of weed seeds. However, some seeds pass through livestock unharmed and can germinate if

spread back onto the land. Preventing weed spread includes controlling weeds around barns and along fences, roads, ditches, and woodlands (Pennstate, 2007).

2.5.2 Mechanical or physical weed management

Physical removal of weeds by soil disturbance prior to planting, and by hoeing and hand-weeding during crop growth are undoubtedly the oldest forms of agricultural weed management. Farmers and agricultural equipment manufacturers continue to develop this ancient tradition of mechanical weed control through the refinement of hand tools and the invention of new tillage and weeding machinery (Matt *et al.*, 2004). Mechanical or physical techniques either destroy weeds or make the environment less favorable for seed germination and weed survival. These techniques include hand-pulling, hoeing, mowing, plowing, disking, cultivating, and digging. Mulching (straw, wood chips, gravel, plastic, etc.) can also be considered a mechanical control means since it uses a physical barrier to block light and impede weed growth (Pennstate, 2007).

From a weed management perspective, tillage re-initiates ecological succession, allowing dominance by early successional annual crops rather than the perennial species that naturally come to dominate undisturbed vegetation (Matt *et al.*, 2001). Cultivation with shallow implements, such as spring-tined cultivators, leaves weed seeds near the surface and encouraged to germinate instead of staying deeply buried and remaining dormant. Deep tillage, such as plowing, buries weed seeds. This strategy helps to eliminate short-lived seeds, since they die while still deeply buried (Final IPM Plan, 1998). Finer seedbeds produce more weed seedlings but a smooth surface makes direct weed control easier (Bond *et al.*, 2003). This method was found to be expensive, exposed the soil to erosion, injurious to superficial roots and ineffective over the long term. Another serious drawback of mechanical methods of weed control was that they were labor-intensive and therefore slow (Philip *et al.*, 2002).

2.5.3 Biological weed management

Biological control of weed is the suppression of weeds by insects and microorganisms that feed on the target plants or otherwise parasitize them (Philip *et al.*, 2002). Biological control would appear to be the perfect solution for pest, disease and weed control in organic and conventional agriculture. In its widest sense it has been taken to include such

basic practices as crop rotation but the term biological control is now usually restricted to the deliberate application of some natural control agents. There is considerable potential for encouraging the use of native bio-control agents against weeds (Liebman and Davis, 2000). Biological weed control involves the use of other living organisms, such as insects, diseases, or livestock, for the management of weed populations (Ananata, 2002; Philip *et al.*, 2002; Pennstate, 2007). The tadpole, ducks etc. are used for weed control in Japan, as in the grass carp in Indonesia and the apple snails in Taiwan (Ananata, 2002).

In theory, biological control is well suited for an integrated weed management program. However, the application of biological weed control in agricultural systems has proved difficult (Bond *et al.*, 2003). The limitations of biological control are that it is a long-term undertaking, its effects are neither immediate nor always adequate, only certain weeds are potential candidates, and the rate of failure for past biological control efforts has been fairly high. There have been a few success stories of weed species being managed with insect or disease bio-control agents. Herbivores such as sheep and goats can provide successful control of some common pasture weeds (Pennstate, 2007).

According to Philip *et al.* (2002), while successful bio-control is extremely economical, success is not assured. Efforts on gorse, faya tree, christmasberry, and others have not been fruitful so far. In addition, because bio-control is species-specific and there are a couple of hundred serious weed species and only limited resources to do the exploratory, safety, and efficacy research, bio-control has to focus on only a few of the most serious weeds. Martin *et al.* (2002) stated that, sometimes, even when we have tried to conserve natural enemies, they are still not effective enough to prevent economic damage. In this situation it is sometimes possible to boost the populations of natural enemies that are already in the ecosystem, by mass rearing them in laboratories or rearing units and then releasing them into the field. This approach to bio-control (augmentative biological control) is not widely used for coffee.

Disadvantages of biological weed control include a long period of five to ten years to achieve control, requires government support, requires high initial investment, and the technology cannot be sold (particularly classical biological control agents). Besides, biological control does not attract industries except myco-herbicides and not suitable for

fast acting short-term control. In addition, use of classical biological control agents may cause non-target effects, regulatory burden and conflict of interest on the import/export of exotic enemies (Taye, 2007). Sometimes, there simply are no natural enemies that are effective against a pest in a particular system. This situation usually arises when exotic pests have been introduced to a new region leaving their natural enemies behind and as a result their numbers increase rapidly. Although the idea is simple, a substantial amount of research and money is involved in developing this type of biological control (Martin *et al.*, 2002). Recently there has been interest in myco-herbicides, pathogenic fungi that are sprayed onto weeds, but in contrast to classical bio-control, these fungi are unable to persist in the environment and die out with the weed (Philip *et al.* 2002).

2.5.4 Weed management by herbicides

The use of chemicals for controlling weeds started with the introduction of 2, 4-D in 1940's. The usage of herbicide is higher than any agro-chemicals. Farming has now realized the importance of herbicide usage for harvesting higher crop yields (Ananata, 2002). Herbicides are often the most cost-effective way of long-term weed control but if misused they carry a greater environmental and pollution risk which has to be taken into account. The usual method of weed control is to target a 1 to 1.5 m spot or band around the tree. Sometimes, on areas of land heavily infested with established weeds or where fast establishment of trees is required, total weed control is needed, but this is a rare occurrence. After establishment, grasses and wildflowers can re-colonize the spot or band to improve the biodiversity and appearance of a site (Anonymous, 2006).

2.5.4.1 Inorganic herbicides

Pesticides present the only group of chemicals that are purposely applied to the environment with aim to suppress plant and animal pests and to protect agricultural and industrial products (PANE, 2010). Herbicides provide a convenient, economical, and effective way to help manage weeds. They allow fields to be planted with less tillage, allow earlier planting dates, and due to reduced tillage, soil erosion has been reduced (Mohan *et al.*, 2011). However, the majority of pesticides are not specifically targeting the pest only and during their application they also affect non-target plants and animals. Many pesticides are not easily degradable, they persist in soil, leach to groundwater and surface water and contaminate wide environment. Depending on their chemical properties

they can enter the organism, bioaccumulate in food chains and consequently influence also human health. Overall, intensive pesticide application results in several negative effects in the environment that cannot be ignored (PANE, 2010). It has been estimated that about 2.5 million tons of pesticides are used on crops every year and the worldwide damage caused by pesticides reaches \$100 billion annually (Mohan *et al.*, 2011).

Pesticides enter the soil via spray drift during foliage treatment, wash-off from treated foliage, release from granulates or from treated seeds in soil. Some pesticides such as soil fumigants and nematocides are applied directly into soil to control pests and plant diseases presented in soil. The transport, persistence or degradation of pesticides in soil depends on their chemical properties as well as physical, chemical and biological properties of the soil. All these factors affect sorption/ desorption, volatilisation, degradation, uptake by plants, run-off, and leaching of pesticides (PANE, 2010). Fungicides were found to be toxic to soil fungi and actinomycetes and caused changes in microbial community structure (Pal *et al.*, 2005).

Glyphosate, nonselective herbicide, and chlorpyrifos, insecticide, belong to the most worldwide used pesticides, especially on transgenic resistant crops. An integrated study on a Roundup resistant soya field in Argentina showed deleterious effect of these pesticides on earthworm population. Earthworms avoided soil with glyphosate; their feeding activity and viability were reduced. Glyphosate and chlorpyrifos caused also several adverse effects at cellular level (DNA damage) that indicated physiological stress (Casabé *et al.*, 2007). Authors also reported that the effects observed on the reproduction and avoidance caused by glyphosate could contribute to earthworm decrease, with the subsequent loss of their beneficial functions. As reported in another study, glyphosate affected predatory arthropods (spiders and ground beetle) in agricultural field, caused behavioural changes and influenced long-term surviving even in residual exposure. These Results also suggest that herbicides can affect arthropod community dynamics separate from their impact on the plant community and may influence biological control in agroecosystems (Evans *et al.*, 2010).

Glyphosate has extremely low volatility, which means that it will not evaporate from surfaces such as soil or treated plants and move through the air after application to injure non-target vegetation. It is the active ingredient in Roundup and Mamba 360 SL

agricultural herbicides may have activity against some fungal pathogens of agricultural crops (Anonymous, 2002). The principal negative effects of glyphosate injury to coffee plants include stunting and arrested growth of plants, nutritional deficiency symptoms (foliar yellowing), poor coffee bean yields, and predisposition to other diseases such as *Cercospora* leaf spot and berry blotch. Banana moth injury to coffee verticals after pruning, and root rots in wet soils after severe coffee pruning, also cause plant damage and slow re-growth of new vertical branches (Nelson, 2008).

Herbicides may not be a necessity on some farms or landscape settings, but without the use of chemical weed control, mechanical and cultural control methods become that much more important. There are many kinds of herbicides from which to choose. Many factors determine when, where, and how a particular herbicide can be used most effectively and knowing these factors enables us to use herbicides to their maximum advantage (Pennstate, 2007). After severe setback arising from the use of chemical pesticides on living systems and the environment, the use of eco-friendly biopesticides is gaining momentum (Mohan *et al.*, 2011).

2.5.4.2 Organic herbicides

In organic farming systems, the aim is not necessarily the elimination of weeds but their management, and organic farming avoids the use of synthetic herbicides which, like pesticides, leave harmful residues in the environment (HDRA, 1998). Naturally green concept suggests the avoidance of use of any pesticide via public education and awareness-raising program, and also to inform public about the potential risk of pesticide use and alternatives that are available. Use of essential oils or their components add to this natural concept owing to their volatility, limited persistence under field conditions (Mohan *et al.*, 2011). Natural products or eco-friendly pesticides are an excellent alternative to synthetic pesticides as a means to reduce negative impacts to human health and the environment. Due to their non-persistent nature, essential oils are considered to be safe to natural enemies (predators and parasitoids) and pose little threat to the environment or human health (Isman, 2008). The move towards green chemistry processes and the continuing needs for developing new crop protection tools with novel modes of action makes discovery and commercialization of natural products as green pesticides, an

attractive and profitable pursuit that is commanding attention. Essential oils are such natural products that have received the interest of researchers.

Essential oils are defined as any volatile oil(s) that have strong aromatic components and give distinctive odor, flavor or scent to a plant. These are the by-products of plant metabolism and are commonly referred to as volatile plant secondary metabolites. They are found in glandular hairs or secretory cavities of plant-cell wall and are present as droplets of fluid in the leaves, stems, bark, flowers, roots and/or fruits in different plants. Plant essential oils are produced commercially from several botanical sources, many of which are members of the mint family (Lamiaceae) (Koul *et al.*, 2008). The oils are generally composed of complex mixtures of several constituents (Miresmailli *et al.*, 2006) such as monoterpenes, diterpens, sesquiterpens and biogenetically related phenols. Examples include 1,8-cineole, the major constituent of oils from rosemary and eucalyptus; eugenol from clove oil; thymol from garden thyme; menthol from various species of mint; and carvacrol and linalool from many plant species (Koul *et al.*, 2008; Mohan *et al.*, 2011).

Essential oils of aromatic plants are being explored to find out possible herbicides since they do not persist in soil or contaminate ground water and causes little or no mammalian toxicity (Isman, 2000) and also be used as viable weed control technology under organic farming systems (Tworkoski, 2002). In recent years, several organic herbicide products have appeared on the market. These include Weed Pharm (20% acetic acid), C-Cide (5% citric acid), GreenMatch (55% d-limonene), Matratec (50% clove oil), WeedZap (45% clove oil + 45% cinnamon oil), and GreenMatch EX (50% lemongrass oil). These products are all contact-type herbicides and damage any green vegetation they come contact with, though they are safe as directed sprays against woody stems and trunks. These herbicides kill weeds that have emerged, but have no residual activity on those emerging subsequently. Additionally, these herbicides can burn back the tops of perennial weeds, but perennial weeds recover quickly (Lanini, 2010). In spite of huge potential for the use of essential oils as safe and effective alternative means of weed control, their application in organic coffee production in the world and in Ethiopia in particular received no attention to date. This definitely calls for concerted effort in determining the efficacy of different essential oils and optimizing their extraction and application.

2.6 Description of essential oil bearing plants

Cymbopogon winterianus: commonly called citronella grass, "Teji sar" in Amharic

C. winterianus is an aromatic grass belonging to the Poaceae family which gives essential oils upon stem distillation. This is used extensively as a source of perfumery, soap, cosmetic and flavoring industry throughout the world. India has been a leading producer of essential oils including oil of citronella (Katiyar *et al.*, 2011). This plant demonstrated depressant effect on the central nervous system, anticonvulsant effect, larvicidal effect against *Aedes aegypti*, antibacterial and antifungal activity, including anti-*Candida* action. The essential oil of *C. winterianus* has concentration-dependent fungicidal activity (Wylly *et al.*, 2011). Citronella oil is commonly known for its natural insect repellent properties, though it has many other uses in aromatherapy. This oil is also known as Java citronella and has a fresh, powerful, lemon like scent. The oil can effectively be used in humidifying diffuser for its insect repellent properties. Traditional uses include treatment of fever, intestinal parasites, digestive and menstrual problems (Katiyar *et al.*, 2011).

Rosmarinus officinalis (L.) commonly called rosemary, "Siga metbesha" in Amharic

Rosemary is a member of the mint family Lamiaceae, which also includes many other herbs. The plant is native to the Mediterranean area, now cultivated widely in different parts of the world, although it thrives in a warm and relatively dry climate. Traditionally, rosemary has been used by herbalists to improve memory, relieve muscle pain and spasm; stimulate hair growth, and support the circulatory and nervous systems; and recently in the prevention of cancer and its antibacterial properties (Al-sereiti *et al.*, 1999). Similarly, *R. officinalis* is also well-known for its substantial insecticidal activities against a number of insect pests in storage structures. It was reported that rosemary essential oil have contact and fumigant toxicity towards *O. surinamensis*, *R. dominica*, *S. oryzae* and *T. castaneum* (Shaaya *et al.*, 1997).

Trachyspermum ammi (Sprague) popularly called as Bishop's weed, "Nech Azmud" in Amharic.

Bishop's weed is a smooth or slightly hairy-branched perennial shrub belonging to the family Apiaceae, reaching a height of 90 cm. It is an aromatic spice closely resembling

thyme in flavor and is native to Egypt and is now distributed in Mediterranean region, Africa and South-West Asia (Srivastava and Satyanarayana, 2006). Bishop's weed oil is a colourless liquid possessing a characteristic odour of thymol and sharp burning taste (Ravindran and Balachandran, 2004). Mintesnot and Mogessie (1999) indicated that crude extracts of bishop's weed contain antibacterial effect against food-borne pathogens. The methanolic extract of the fruits of this plant yielded a monoterpene with phenolic properties, which when tested in vitro against adult worms of *Setaria digitata*, the filarial parasite of cattle, resulted in more than 80% immobilization of the worms at an incubation period of 2 hours (Srivastava and Satyanarayana, 2006).

Eucalyptus globulus (Labill), "*Nech bahirzaf*" in Amharic

E. globulus is one of the most widely cultivated of Australia's native trees, belonging to the family Myrtaceae. It is a medium to very tall forest tree, which may reach 70 m in ideal conditions but is more commonly 15-25 m in height. Leaves are mostly curved, acuminate at tip, and thick and leathery entire surface with fine straight veins and vein inside marlin, shiny dark green on both surfaces. It was reported to have a number of medicinal values against diseases of abscess, arthritis, asthma, boils, bronchitis, burns, malaria, cold, cough, croup, and the like (Jones, 1998). Moreover, leaf powder, essential oil and solvent extracts of the plant part have been noted to have the potential of using as insect pest control against *S. oryzae* (L.), *Stegobium paniceum* (L.), *T. castaneum*, *C. chinensis*, *C. maculata* and *A. obtectus* (Papachristos and Stamopoulos, 2002; Lee *et al.*, 2003; Papachristos *et al.*, 2004). For instance, the oil of *E. globulus* was reported to have contact toxicity and a seed protection effect against the pulse beetle (Srivastava *et al.*, 1988). Negahban and Moharramipour (2007) evaluated fumigant toxicity of different *Eucalyptus* species.

Eucalyptus citriodora also called lemon-scented gum, blue spotted gum and lemon eucalyptus

E. citriodora is a plant native to Australia, and is widely cultivated around the world and belongs to the family Myrtaceae (Dawar *et al.*, 2007). Muhammad *et al.* (2008) reported that *Eucalyptus* species release volatile compounds such as benzoic, cinnamic and phenolic acids which inhibit growth of crops and weeds growing near to it. Herbicidal

activities of volatile oils of *E. citriodora* against *P. hysterophorus* L. has been reported by Singh *et al.* (2005). Among various species of eucalyptus, lemon-scented eucalypt is well-known for its antimicrobial, antifungal, and insecticidal activities (Isman, 2000). *E. citriodora* have very little vegetation under their canopy and around them and it is due to the release of oil vapours from the trees, which move downwards and affect the adjoining vegetation (Setia *et al.*, 2007).

***Artemisia annua* L.** commonly called annual wormwood, sweet wormwood

A. annua is originated from China and widely distributed in the temperate, cool temperate and subtropical zones (mainly in Asia) of the world. However, it also grows in the Mediterranean region and countries in North Africa, as well as in south and south-west Asia. In addition, following its settlement in North America from northern Asia, it grows widely in Canada and the United States (Anonymous, 1991). A few countries are currently cultivating *A. annua* on a large scale, such as China, Kenya, the United Republic of Tanzania and Viet Nam. *A. annua* for industrial use is mainly collected from the wild (WHO, 2006). It is annual plant, aromatic, green, glabrous or with scattered, small, approximate hairs, while; stem erect, ribbed, brownish or violet-brown, naturally grows to 30-100 cm high (Shishkin and Bobrov, 1995) and belonging the family Asteraceae (Jorge and Jules, 2009). *A. annua* traditionally used in China to treat fevers and hemorrhoids. Duke *et al.* (1987) reported the non-selective herbicide effectiveness of this crop.

***Piper nigrum* L.** commonly called black pepper

P. nigrum is a climbing plant belonging to the family Piperaceae. It is originally from Southeast Asia, specifically India and it is the most common and important of the spices (Costa *et al.*, 2011). *P. nigrum* is perennial glabrous woody climber that grows to a height of 10 m or more, clinging to its support and widely used as condiments, and has extensive culinary uses, and is used in meats, soups, fish, pickles, and ketchups. Moreover, this spice is also used for medicinal purpose, as preservative, as flavourant in culinary seasonings, and the oil, the pungent character and the oleoresin have pharmaceutical, perfumery, and etc. uses (Girma *et al.*, 2005). Ravi and Gayatri (2007) reported *P. nigrum* oil was shown significant repellent activity at 0.2 percent concentration on *T. castaneum* and Fan *et al.* (2011) also reported insecticidal activities of this crop on larvae of *S. litura*.

***Ricinus communis* L.** commonly known as castor oil plant, "*Gulo*" in Amharic

The castor bean plant originated in Ethiopia and gradually dispersed towards South Africa, the Mediterranean region and warm areas of Asia, until finally establishing itself as a natural species in the majority of warm climate regions of the world (Garcíaa-Gonzaâ Lez *et al.*, 1999). This plant belongs to the Euphorbiaceae family, and is a very large, rapidly growing, bush (reaching heights of up to 5 m), with a woody, hollow stem (Garcíaa-Gonzaâ Lez *et al.*, 1999; Sylvia *et al.*, 2011). *R. communis* is well known for its biological activities; most important of which are, hepatoprotective, laxative, antidiabetic, and antifertility activities. Its seeds yield castor oil, which has been used to treat liver infections and to cure inflammation in Indian system of medicine (Sumera *et al.*, u.d). Castor seeds are a rich source of oil which can be extracted by milling, boiling, pressing or solvent extraction (Sylvia *et al.*, 2011). Tounou *et al.* (2011) reported that castor bean plant extracts and oil could be toxic to larvae of *P. xylostella* through contact and ingestion.

3. MATERIALS AND METHODS

3.1 Description of the Study Area

The first two experiments (laboratory bioassay and lathhouse experiment) were conducted at Jimma University College of Agriculture and Veterinary Medicine (JUCAVM), Jimma, Ethiopia from October, 2011 to February, 2012. JUCAVM is geographically located at 346km southwest of Addis Ababa, lies at an elevation of 1710 meters above sea level and 7^o, 33' N latitude and 36^o, 57' E longitude. The mean annual rainfall of the area ranges from 1200 to 2000 mm. Within a year, the maximum temperature ranges from 25^oC to 30^oC from January to April while the minimum ranges from 7^oC to 12^oC, from October to December (BPEDORS, 2000). The soil in the study area is characterized as clay-loam in texture with an organic matter content of 3 to 5% and a pH of 5.6 to 6.5, which is considered as the best soil for coffee growing.

The third experiment was conducted at Jimma Agricultural Research Center (JARC), Jimma, Ethiopia from June to September, 2012. JARC lies at an elevation of 1753 meters above sea level and 7^o, 46' N latitude and 36^o, 0' E longitude. The average annual rainfall of the area is about 1500 mm per annum with minimum and maximum temperatures of 11.8^oC and 26.2^oC, respectively, and relative humidity of the area is 67% based on long-term Metrological data of the center (1968 to 2006) (Girma *et al.*, 2008). The soil group of the experimental site is Nitosol or Eutric Nitosol according to FAO soil classification. It is characterized by reddish-brown or dark-brown clay or clay loam texture, well drained and with gentle slope. It is slightly acidic with pH ranging from 5.7 to 6.0 (Solomon *et al.*, 2008).

3.2 Pre-experimental Conditions

3.2.1 Collection of plant materials

The samples of test plants were collected mainly from Addis Ababa and Wendogenet Research Center. The seeds of Caster plants were collected around Melkasa Agricultural Research Center, whereas, inert materials were gathered around Adame Tulu Pesticide Formulation Company Ltd. Analytical grades of H₂O₂ was bought from chemical vendors (Table 1).

Table 2: Plant materials the extract of which having herbicidal activity on weed species of coffee

Scientific name	Common name	Description
<i>Eucalyptus citrodora</i>	Eucalyptus	Herb
<i>Cymbopogon winterianus</i>	Citronella	Herb
<i>Eucalyptus globulus</i>	Eucalyptus	Herb
<i>Trachyspermum ammi</i>	Bishop weed	Seed
<i>Artemisia annua</i>	Wormwood, anis	Herb
<i>Piper nigrum</i>	Black pepper	Herb
<i>Rosmarinus officinalis</i>	Rosemary	Herb
<i>Ricinus communis</i>	Castor-Oil Plant	Seed
Inert material A	Bole	Powder
Inert material B	Diatomate	Powder
Hydrogen per oxide	H ₂ O ₂	Liqued

3.2.2 Plant extraction and formulation methods

The essential oils of the test plant parts were extracted by hydro-distillation using Clevenger Apparatus in the Crop Quality Laboratory of EIAR, Addis Ababa. 100g amount of these fresh plant materials were cut into small pieces and placed in different distillation flask contained 200ml distilled water. Each distillation flask was placed in boiling water (100⁰C) for three hours. When the process of distillation was completed, essential oils were collected; measured and percent oil content per biomass was calculated for analytical purposes. The distillate was collected in a separating funnel in which the aqueous portion was separated from the volatile oil. The water was slowly discarded until only the oil layer remained. Thus, the oils were collected in a plastic container and stored at low temperature (4⁰C) in a refrigerator until it was used for all experiments. On the other hand, inert materials were activated by drying under reflux and extracted using Soxhlet apparatus. Oils of Caster bean was extracted with hexane using Soxhlet apparatus, 100g grinded seed/200ml hexane. The oil was dried using rotary vacuum and dried in oven (40⁰C) for overnight. The powder of Bole and Diatomate with a mass of 30g for each was diluted with 70ml of 70% ethanol separately and filtered with filter paper (Whatman No. 1) then the concentrate was diluted for application. 30ml of Hydrogen per oxide was also diluted with 70ml of ethanol before used. Ethanol (70%) was used to prepare stock solutions from each oil and subsequent dilutions to appropriate concentrations ranging from 0.03, 0.06 and 0.09% (v/v) for laboratory and 5% (v/v) for lathhouse and field experiments.

3.3 Treatments, Experimental Design and Procedure

3.3.1 Inhibitory of botanicals on *B. pilosa* under laboratory conditions

A laboratory experiment was conducted to study the effect of selected botanicals against *B. pilosa* seed germination and early growth parameters. Thirty five treatments (eleven different treatments, i.e. plant extracts and inert minerals combined each with three concentration levels, and for comparison a standard check and control) were used for the experiment. The test weed, botanicals and other materials included *Artemisia annua* L. (Leaves), *Rosmarinus officinalis* (Aerial parts), *Trachyspermum ammi* (Seeds), *Cymbopogon winterianus* (Leaves), *Eucalyptus citrodora* (Leaves), *Eucalyptus globules* (Leaves), *Piper nigrum* (Seeds), Diatomate (Powder), Bole (Powder), *Ricinus communis* (Seeds) and H₂O₂ (Liquid). *B. pilosa* was used as a test weed. The experiment was laid out in a 11 x 3 factorial arrangement in Completely Randomized Design with three replications and a total of 105 experimental units.

The seeds of *B. pilosa* were surface sterilized by shaking them for five minutes in 1% sodium hypochlorite (NaOCl) solution and washed with de-ionized water for three minutes immediately before use to remove all chemical residues and fifty seeds were sown maintaining equidistance per sterilized Petri dish that had a diameter of 9 cm and lined with double layer of filter paper (Whatman No. 1). Then 10 ml oil and inert mineral of desirable concentrations (0.03, 0.06 and 0.09% (v/v)) was poured on the filter paper and allowed to diffuse by covering the plates. The standard check and control treatments received 10 ml of the recommended Mamba 360 SL and distilled water respectively. The experiment was placed on a laboratory table under the average maximum and minimum temperature of 22.4 and 19.5⁰C with the relative humidity ranging from 47.2 to 87.2% during the experiment period and the Petri dishes were examined for seed germination regularly. The experiment was conducted twice, germinated and non-germinated seeds counted, and the average data were used for analysis.

3.3.2 Herbicidal activity of botanicals on *B. pilosa* under lathhouse conditions

This investigation was conducted in the lathhouse of the JUCAVM, Jimma, Ethiopia, during the summer season of 2011/12. Thirty five treatments (eleven different treatments, i.e. plant extracts and inert minerals combined each with three application frequencies, and for comparison a standard check and control) were used for the experiment. The test weed, botanicals and other materials included *Artemisia annua* L. (Leaves), *Rosmarinus officinalis* (Aerial parts), *Trachyspermum ammi* (Seeds), *Cymbopogon winterianus* (Leaves), *Eucalyptus citrodora* (Leaves), *Eucalyptus globules* (Leaves), *Piper nigrum* (Seeds), Diatomate (Powder), Bole (Powder), *Ricinus communis* (Seeds) and H₂O₂ (Liquid). Mamba 360 SL and Seedlings of *B. pilosa* were used as standard check a test weed, respectively. The experiment was laid out in a 11 x 3 factorial arrangement in Randomized Complete Block Design with three replications and a total of 105 experimental units.

Sieved agriculture soil was obtained from JUCAVM and used for the pot experiment in lathhouse. The pots, which had a 20 cm diameter and 20 cm height, contained equal amounts of sieved soil. Seeds of *B. pilosa* were surface sterilized by shaking them for five minutes in 1% sodium hypochlorite (NaOCl) solution and washed with de-ionized water for three minutes immediately to remove all chemical residues. Twenty seeds per pot were sown 2 cm deep into the soil and grown under the average maximum and minimum temperature of 24.5 and 11.5⁰C. The relative humidity ranged from 26.6 to 78.5% during the experiment period. Routine watering was done and the seedlings were thinned out, so that only 20 homogeneously grown seedlings were left per pot. All the plant extracts and inert minerals were applied each at concentration of 5% (v/v) 1x, 2x and 3x as foliar spray at 50, 54 and 58 days after sowing while the standard check was sprayed as per recommended rates once at 50 days after sowing. The volume of each treatment was diluted using distilled water and weed plants/pot received the volume of 20 ml per pot by spraying using hand sprayers and equal volume of distilled water was sprayed as control treatment.

3.3.3 Herbicidal effects of botanicals on common weeds of coffee at field trial

The field trial was conducted at the experimental site of the JARC, Jimma, Ethiopia, during the rainy season of 2012 from June to September. Eleven treatments (three different plant extracts, combined each with three application frequencies and for comparison a standard check and control) were used for the experiment. The test weeds and botanicals included *Trachyspermum ammi* (Seeds), *Cymbopogon winterianus* (Leaves) and *Eucalyptus citrodora* (Leaves). Common coffee weeds were used as a test weeds. The experiment was laid out in a 3 x 3 factorial arrangement and standard check applied once as recommended, in Randomized Complete Block Design. Each treatment was replicated three times and a total of 33 experimental units were compared. The gross plot size of the experiment was 6m² (2m x 3m) whereas the distance between plots was 2m. All the three plant extracts were applied each at concentration of 5% (v/v) 1x, 2x and 3x as foliar spray using hand sprayers at 30, 34 and 38 days after uniformly slashing the weeds in the study site while the standard check was sprayed as per recommended rates once at 30 days after slashing. The volume of each treatment was adjusted using distilled water and each plot received the solution (200 ml per plot) and Mamba 360 SL used as standard check.

3.4 Data Collection, Computation and Analysis of Variances

3.4.1 Data collection and computation

During the Petri dish bioassay, germinated seeds were counted daily from the second to fifteenth day after sowing. Counting was stopped on the fifteenth day because the number of germinated seeds or emerged seedlings was constant after this day.

Germination percentage (%): Germination percentage is the number of seeds that produce a seedling from a seed population and expressed as a percentage. When the seed germination become stable, the count was terminated and germination percentage was calculated as prescribed by ISTA (1993). The per cent germination was expressed on normal seedling bases.

$$\text{Germination percentage (\%)} = \frac{\text{Number of normal seedlings}}{\text{Total number of seed sown}} \times 100$$

Germination/Emergence Rate (G/ER): Germination/Emergence rate is a measure of the speed or velocity of germination of seeds germinated or seedlings emerged per day from the seeds sown per Petri dish. It shows the number of seeds that germinate on average per day and calculated by the formula proposed by Maguire (1962) as:

$$\text{G/ER (seeds per day)} = \frac{\text{Number of normal seedlings}}{\text{Days of first count}} + \dots + \frac{\text{Number of normal seedlings}}{\text{Days of final count}}$$

Plumule and radicle lengths: Plumule and radicle lengths were performed visually using a ruler (direct measurement) on the fifteenth day after seed sowing from five randomly selected normal seedlings. Average of five plumule and radicle lengths were calculated separately and expressed in centimeter. The inhibitory or stimulatory percent was calculated using the following equation modified from Chung *et al.* (2001) as:

$$\text{Inhibition (-) or stimulation (+) percentage (\%)} = \left(\frac{\text{Treatment} - \text{Control}}{\text{Control}} \right) \times 100$$

Shoot length and root length: In lathhouse experiments, *B. pilosa* seedlings were treated under different treatment conditions 50 days after sowing. Fourteen days after treatment, five randomly selected normal seedlings were taken and separated into shoot and root parts. The roots were washed with tap water to remove the soil load and measurements of shoot and root lengths were determined using a ruler (direct measurement). Average of five shoot and root lengths were calculated and expressed in centimeter.

Above and underground biomass: Root and shoot weights of the randomly selected five normal seedlings that used for measuring root and shoot lengths were used for recording fresh as well as dry weight of seedlings and quantified in grams. Root and shoot dry weights (g) were determined after drying in an oven (DP203A, China) maintained at 70⁰C until two consecutive measurements had no difference. Fresh as well as dry weights (g) of the five sampled plants were measured using sensitive balance (CTG-6H⁺, USA).

For field experiment, weeds were treated at succulent stage, but before flowering and most of the sources of data were conceded with the population/biomass of weed species.

Weed species (count number): Within a plot, weed density was taken by using a quadrant having a size of 50cm x 50cm from two focal points (under and out shade), since type as well as population of weeds at these two places were not the same. All weeds within a quadrant were identified (broadleaf or grassy) and counted before applying the respective treatments, then fourteen days after treatment, weeds in each quadrant were again identified and counted to know type of weeds inhibited and/or survived and the result was expressed by percentage.

The date for the first occurrence of visible symptoms like color change and wilting of *B. pilosa* under lathhouse and common weed species of coffee under field condition was detected by personal visual observation. According to European Weed Research Council (EWRC) scoring system, death percentage was assessed by using scoring methods (on scale of 1 to 9), 1 being for 100% weed control and 9 for no effects. Damaged parts (leaf, branch, stem, etc) of weeds were also examined and the findings are reported accordingly.

Total (above and underground) biomass: Weeds in a quadrant from both places were separately uprooted by digging the area of quadrant to the depth of 15cm, because majority of root of weeds are found close to superficial area and may not go beyond this depth. The roots were washed with tap water to remove the soil load and measurements of fresh weight of all weeds within a quadrant were separately measured. Dry weight of weeds was recorded after drying in an oven (DP203A, China) maintained at 70⁰C temperature until two consecutive measurements had no difference. Fresh as well as dry weights of the sampled weeds within quadrant were measured using sensitive balance (CTG-6H⁺, USA) and expressed in grams.

3.4.2 Data analysis

The validity of model assumptions was verified by examining the residuals for each of the response variables as outlined in Montgomery (2008). For some response variables square-root transformation method was needed to meet the normal distribution of the error terms assumptions and mean separation was done using the LSD calculated from the transformed data. Accordingly, Square transformed $(X+0.5)^{1/2}$, where X is the original data of germination percentage, germination rate, shoot and root length were performed by using SAS version 9.2. (SAS Institute Inc., 2008). Two phases of statistical analysis were employed. The first one involved determining whether the main and/or interaction effects of the fixed factors of interest were significant. The second one involved further analysis to compare the least significance means of the treatment combinations of significant interactions.

In order to avoid the influence of unequal level of concentration/frequency of application, all data for each parameter were analyzed in two phases, first factorial in 3 replications/blocks with the exclusion of control and standard check treatments. Hence, the three levels of concentrations for laboratory and once, twice and three times application frequency, for lathhouse and field experiments, were used as fixed factors of interest in the model. When the interaction effect of the fixed factors (plant extraction and concentration/application frequency) was significant ($P < 0.05$), data was analyzed by two-way Analysis of Variance (ANOVA) and computed using SAS version 9.2. (SAS Institute Inc., 2008). For significant parameters, multiple comparisons of means were conducted to separate the means of significant fixed effects including control and standard check by using LSD (Least Significance Difference) test. Simple correlation analysis was also performed in order to see the relationship among the response parameters.

4. RESULTS

The results of the experiment conducted to study the herbicidal effects of botanicals (plant extract and natural activated inert materials) on *B. pilosa* weed under both laboratory and lathhouse circumstances and on common weed species of coffee in the farm during 2011/2012 cropping season at JUCAVM and JARC, Jimma, Southwestern Ethiopia. The result of these experiments is presented in this particular chapter.

4.1 Bioassay inhibitory effects of tested materials against *B. pilosa*

4.1.1 Inhibitory effect on seed germination

Seed germination measured as percent (%) fifteen days after seed sowing was influenced by types of essential oils and their concentration. The result revealed that all plant extracts and naturally activated inert materials suppressed the germination of the seed of tested weed in a Petri dish and the inhibitory potential of the essential oils, was observed to vary with the sources of botanical species as well as each individual concentration with statistically very highly significant ($P < 0.001$) variation (Figure 1 and Appendix Table 1). Higher concentration (0.09% (v/v)) of botanicals and inert minerals applied exhibited correspondingly high inhibitory effect on seed germination as compared to lower concentration (0.03% (v/v)) (Appendix Figures 1, 2 and 3). The minimum seed germination status (2%) was obtained as a result of treatment with *E. citrodora* and *C. winterianus* in which the level of seed germination inhibition was found attained 97.96% at 0.09% (v/v) concentration; whereas the maximum seed germination (98%) was observed due to the untreated (control) followed by treatment with H_2O_2 (86.67%) at 0.03% (v/v) concentration.

At concentration of 0.09% (v/v), except treatments of *P. nigrum* extract, Bole and H_2O_2 , the inhibitory effects on seed germination for all treatments was higher than that of the standard check. Likely; at concentrations of 0.03 and 0.06% (v/v), the inhibitory effects for all treatments were lower than standard check except for essential oils from *C. winterianus*, *T. ammi* and *R. communis*. Even though no significant differences statically were observed, germination percent was low at concentration of 0.06% (v/v) for *T. ammi* and *R. communis* treatments than that of standard check. The seed treated with H_2O_2 at all

tested concentration levels (0.03, 0.06 and 0.09% (v/v)) resulted in high germination percentage as compared to other treatments, but lower than untreated control.

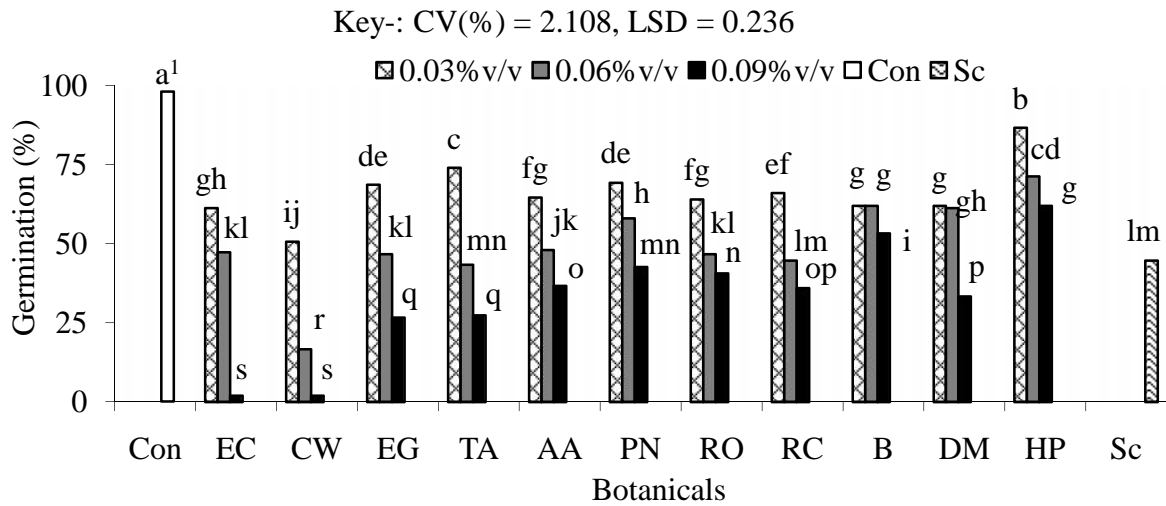


Figure 1: Mean seed germination percentage of *B. pilosa* as influenced by botanical extracts and inert minerals

¹Means followed by different alphabet(s) differ significantly at ($P < 5\%$)

NB:- Con = Control, EC = *E. citrodora*, CW = *C. winterianus*, EG = *E. globules*, TA = *T. ammi*, AA = *A. annua*, PN = *P. nigrum*, RO = *R. officinalis*, RC = *R. communis*, B = Bole, DM = Diatomate and HP = Hydrogen Per oxide, Sc = Mamba 360 SL

4.1.2 Inhibitory effect on seed germination rate

Results relating to speed of germination are depicted by Figure 2 and Appendix Table 1. Data pertaining to the effect of type of essential oils and inert minerals with their concentration on germination rate were analyzed and the results showed very highly significant ($P < 0.001$) variations. All the applied concentrations of each botanical extracts and inert minerals significantly suppressed the seed germination rate of the tested weed. The inhibitory potential of the essential oils and inert minerals, however, was found to vary very highly and significant ($P < 0.001$) with the sources of botanical species and minerals as well as their individual concentration. Accordingly, at a concentration of 0.09% (v/v), germination rate for seeds treated with some essential oils and inert minerals was very slow as compared to lower concentrations (0.03 and 0.06% (v/v)) and the untreated control. The rate of germination was faster for seeds treated with only distilled water (54.42 seeds per day) followed by those treated with H_2O_2 (40.11 and 32.01 seeds

per day) at concentration of 0.03 and 0.06% (v/v), respectively. The lowest rate was noted from seeds treated with *E. citrodora* and *C. winterianus* (0.24 and 0.16 seeds per day) respectively, at high concentration (0.09% (v/v)). Germination was relatively faster for seeds treated with standard check (21.16 seeds per day) than all treatments except control and that of the H₂O₂ at 0.03 and 0.06% (v/v) concentrations.

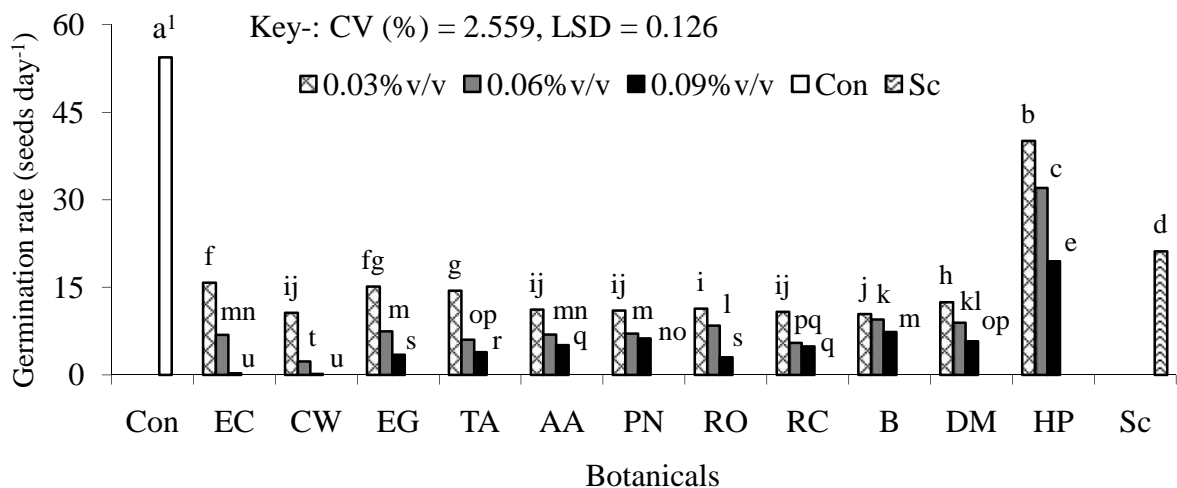


Figure 2: Mean rate of seed germination of *B. pilosa* as affected by botanical extracts and inert minerals

¹Means followed by different alphabet(s) differ significantly at ($P < 5\%$)

NB:- Con = Control, EC = *E. citrodora*, CW = *C. winterianus*, EG = *E. globules*, TA = *T. ammi*, AA = *A. annua*, PN = *P. nigrum*, RO = *R. officinalis*, RC = *R. communis*, B = Bole, DM = Diatomate and HP = Hydrogen Per oxide, Sc = Mamba 360 SL

4.1.3 Inhibitory effect on plumule length

Plumule length measured from randomly selected five normal seedlings after fifteen days of sowing was highly influenced by types of essential oils and inert minerals with their concentration ($P < 0.001$) (Figure 3 and Appendix Table 1). From the data it was evident that there was a decrease in seedling length as tested concentrations increased from 0.03 to 0.09% v/v for all botanicals and inert minerals and the variations among the treatments were statistically very highly significant ($P < 0.001$) except for essential oils from *T. ammi*, *A. annua* and H₂O₂, which showed statistically indifference at 0.06 and 0.09% (v/v) concentrations. Plumule length taken after fifteen days of sowing revealed that the tallest

seedlings (3.37cm) were obtained as a result of treatment with distilled water followed which was by H₂O₂ and Bole (3.21 and 3.10 cm) respectively, at 0.03% (v/v) concentration, even though there appeared statistically no difference; whereas the shortest seedlings were observed from the standard check (0.14 cm) followed by treatments sourced from *C. winterianus* (0.41 cm) at concentration of 0.09% (v/v), which however statically similar.

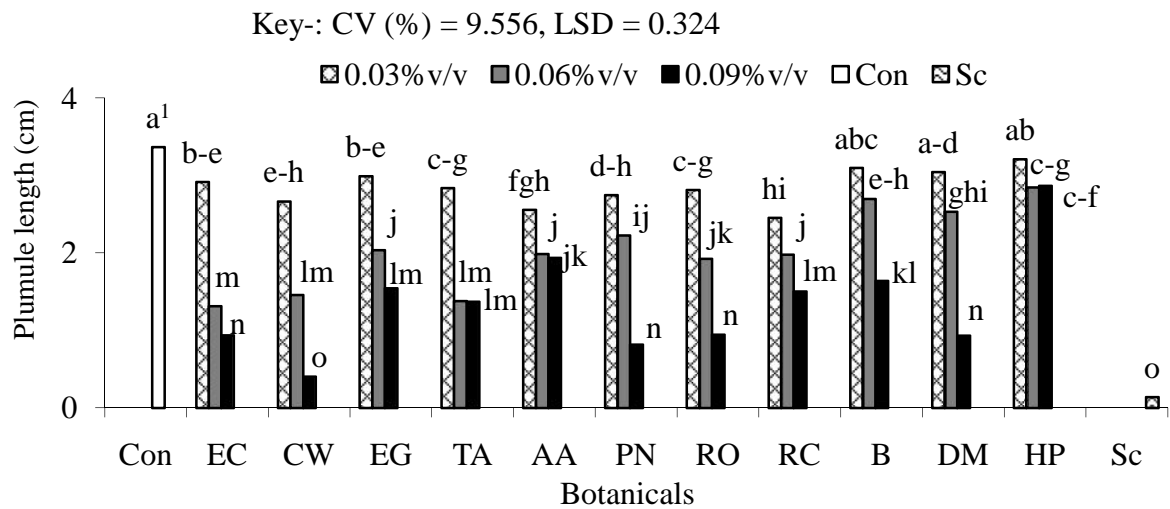


Figure 3: Mean plumule length (cm) of *B. pilosa* as influenced by botanical extracts and inert minerals with different concentrations

¹Means followed by different alphabet(s) differ significantly at ($P < 5\%$)

NB:- Con = Control, EC = *E. citrodora*, CW = *C. winterianus*, EG = *E. globules*, TA = *T. ammi*, AA = *A. annua*, PN = *P. nigrum*, RO = *R. officinalis*, RC = *R. communis*, B = Bole, DM = Diatomate and HP = Hydrogen Per oxide, Sc = Mamba 360 SL

4.1.4 Inhibitory activity against radicle length

Results pertaining to radicle length presented in Figure 4 and Appendix Table 1 depict very highly significant ($P < 0.001$) effect of different treatments. The maximum radicle length (5.13 cm) was obtained as a result of treatment with distilled water followed by *R. officinalis* (4.58 cm) at 0.03% (v/v), which were statistically at par; whereas the minimum was observed from the standard check (0.2 cm) which was followed by application of extract from *C. winterianus* (0.37 cm) at 0.09% (v/v), which however, happened to be statistically similar. Radicle length was appeared to be less inhibited as compared to plumule length for each extracts each with respective concentration.

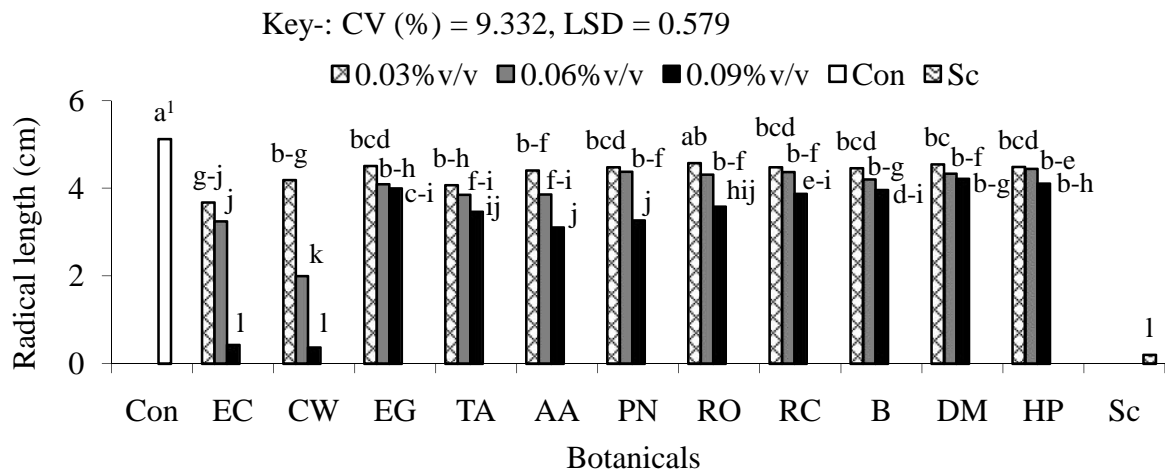


Figure 4: Mean radicle length (cm) of *B. pilosa* as influenced by botanical extracts and inert minerals

¹Means followed by different alphabet(s) differ significantly at ($P < 5\%$)

NB:- Con = Control, EC = *E. citrodora*, CW = *C. winterianus*, EG = *E. globules*, TA = *T. ammi*, AA = *A. annua*, PN = *P. nigrum*, RO = *R. officinalis*, RC = *R. communis*, B = Bole, DM = Diatomate and HP = Hydrogen Per oxide, Sc = Mamba 360 SL

4.2 Efficacy of tested materials against *B. pilosa* under lathhouse condition

4.2.1 Activity against shoot length growth

Results in respect of shoot length of normal seedlings after fourteen days of treatment with essential oils and with their application frequency appeared very highly significant ($P < 0.001$) variation (Table 3 and Appendix Table 2). In contrast to the results obtained under laboratory conditions in Petri dishes, lower inhibitions of tested materials were observed on growth of *B. pilosa* under lathhouse conditions in pot-culture (Table 3). Essential oils extracted from *E. citrodora*, *C. winterianus*, *E. globulus*, and *R. officinalis* reduced shoot length of *B. pilosa* seedlings (-59.70%, -81.75%, -15.73% and -23.02%) as compared to control on average bases, respectively. Signs of injury on *B. pilosa* seedlings were observed within 20-30 minutes after treatment and maximum injury occurred within 24 hour of application of the essential oil of *C. winterianus* and *E. citrodora* in pot experiment (Figure 5) and after two weeks of treatment, no seedlings were observed from pots of the standard check and *C. winterianus* treatments with 2x and 3x applications. Plants with *R. officinalis* and *E. globules* treatments also showed sign of injury at the tip of shoots and leaves, but recovered quickly, on the other hand, sign of inhibition was not observed on other treatments. Instead growth was similar to untreated seedlings (Figure 6).

Similar to the results under laboratory conditions, there was an increase in inhibitory effect with an increase in the frequency of application for few botanical extracts. Evidently, shoot length showed the tendency to decrease as application frequency was increased for treatments of *E. citrodora*, *C. winterianus*, *E. globulus* and *R. officinalis*, and at high application frequency (2x and 3x); *C. winterianus* was on par with standard check (Figure 6). Shoot length taken after two weeks of treating revealed that the tallest seedlings (18.16 cm) was obtained as a result of treatment with *R. communis* followed by H_2O_2 (17.89 cm) on average base, whereas no seedlings were observed from the standard check and *C. winterianus* at 2x and 3x application frequency with maximum (100%) inhibition when compared to untreated control.

Table 3: Effect of botanicals and inert minerals on shoot length of *B. pilosa* under lathhouse conditions at Jimma, Southwestern Ethiopia, in 2011/12

Botanicals	Shoot length (cm)				Efficacy relative to control (%)
	Frequency			Mean	
	1x	2x	3x		
<i>E. citrodora</i>	11.91(3.52h ¹)	7.39(2.81j)	1.77(1.5k)	7.02	59.70
<i>C. winterianus</i>	9.53(3.17i)	0.00(0.71l)	0.00(0.71l)	3.18	81.75
<i>E. globulus</i>	16.97(4.18abc)	15.36(3.98de)	11.71(3.49h)	14.68	15.73
<i>T. ammi</i>	17.63(4.25abc)	17.2(4.21abc)	17.11(4.2abc)	17.31	0.63
<i>A. annua</i>	17.45(4.23abc)	17.17(4.2abc)	17.23(4.21abc)	17.28	0.80
<i>P. nigrum</i>	17.15(4.2abc)	16.34(4.1bcd)	16.01(4.06cd)	16.50	5.28
<i>R. officinalis</i>	14.27 (3.83ef)	13.53(3.75fg)	12.5(3.6gh)	13.41	23.02
<i>R. communis</i>	18.09(4.31a)	18.09(4.31a)	18.29(4.33a)	18.16	4.25(+)
Bole	17.73(4.27ab)	17.91(4.29ab)	17.51(4.24abc)	17.72	1.72(+)
Diatomate	17.26(4.21abc)	17.5(4.24abc)	17.28(4.21abc)	17.35	0.40
H ₂ O ₂	17.86(4.29ab)	17.91(4.29ab)	17.91(4.29ab)	17.89	2.70(+)
Mean	15.98	14.40	13.39	14.59	16.25
Control		17.42(4.23abc)			-
Sc		0.00(0.71l)			100.00
LSD		0.195			
CV (%)		3.183			

¹Means followed by different alphabet(s) differ significantly at ($P < 5\%$)

NB:- 1x, 2x and 3x = Sprayed once, twice and trice, respectively, Sc = Mamba 360SL,

(+) = Stimulated growth

4.2.2 Activity against root length growth

Observation recorded on root length (cm) is presented in Table 4. The data revealed that significant differences among treatments were observed. Root length as influenced by types of essential oils and with their application frequency was analyzed and the result revealed very highly significant ($P < 0.001$) variations (Table 4 and Appendix Table 2). As

compared to the respective control, essential oils from *E. citrodora* and *C. winterianus* highly inhibited root length, while; there was no difference for the rest botanicals and inert minerals. Higher inhibition in root length (100%) was obtained as a result of treating with standard check followed by *C. winterianus* and *E. citrodora* (85.84% and 54.11%) respectively, compared to control on average bases.

Root length taken after two weeks of treating revealed that the tallest seedlings (43.07 cm) was obtained as a result of treatment with Bole followed by *A. annua* (42.65 cm) on average base, even though statistically no difference; whereas no seedlings were observed from the standard check and *C. winterianus* at high application frequency (2x and 3x) with maximum (100%) inhibition when compared to untreated control. Root length tends to decrease as application frequency increased for treatments of *E. citrodora* and *C. winterianus*, and noticeably, at high application, similar to standard check, *C. winterianus* inhibited root length of *B. pilosa* seedlings. While, other botanical extracts recorded least inhibition and root length was less inhibited as compared to shoot length for each extracts and inert minerals with their respective concentration.



Control



C. winterianus



E. citrodora

Figure 5: *B. pilosa* seedlings 20-30 minutes after treating as showed signs of injury by essential oil extracted from *C. winterianus* and *E. citrodora*

Table 4: Effect of botanicals and inert minerals on root length of *B. pilosa* under lathhouse conditions at Jimma, Southwestern Ethiopia, in 2011/12

Botanicals	Root length (cm)				Efficacy relative to control (%)
	Frequency			Mean	
	1x	2x	3x		
<i>E. citrodora</i>	34.31(5.87a ¹)	11.53(2.96c)	11.33(3.01c)	19.06	54.11
<i>C. winterianus</i>	17.65(4.26b)	0.00(0.71d)	0.00(0.71d)	5.88	85.84
<i>E. globulus</i>	44.07(6.68a)	42.71(6.57a)	40.34(6.37a)	42.38	2.05(+)
<i>T. ammi</i>	41.5(6.47a)	42.39(6.54a)	38.2(6.22a)	40.70	2.00
<i>A. annua</i>	43.09(6.6a)	42.63(6.57a)	42.21(6.53a)	42.64	2.67(+)
<i>P. nigrum</i>	43.25(6.61a)	41.35(6.46a)	40.18(6.38a)	41.59	0.14(+)
<i>R. officinalis</i>	41.54(6.47a)	41.22(6.46a)	35.43(5.99a)	39.40	5.13
<i>R. communis</i>	42.04(6.52a)	40.5(6.4a)	40.57(6.41a)	41.04	1.18
Bole	43.16(6.6a)	43.75(6.65a)	42.31(6.54a)	43.07	3.71(+)
Diatomate	42.31(6.54a)	41.59(6.49a)	41.27(6.46a)	41.72	0.46(+)
H ₂ O ₂	41.6(6.48a)	40.56(6.4a)	41.11(6.45a)	41.09	1.06
Mean	39.50	35.30	33.91	36.23	12.76
Control		41.53(6.48a)			-
Sc		0.00(0.71d)			100.00
LSD		0.972			
CV (%)		10.22			

¹Means followed by different alphabet(s) differ significantly at ($P < 5\%$)

NB:- 1x, 2x and 3x = Sprayed once, twice and trice, respectively, Sc = Mamba 360SL,

(+) = Stimulated growth



Figure 6: *B. pilosa* seedlings fourteen days after treating as influenced by botanical extracts and inert minerals with different application frequencies

NB:- 1x, 2x and 3x = Sprayed once, twice and thrice, respectively

4.2.3 Effect of botanical extracts against weed density

Weed density was measured after fourteen days of treatment applications, which was highly affected by types of essential oils and with their application frequency. The data was analyzed and the result revealed that there was very highly significant ($P < 0.001$) variations (Table 5 and Appendix Table 3). The highest (100%) variation relative to control was obtained as a result of treating with standard check followed by *C. winterianus* (92%) on average bases. As application frequency of essential oils from *E. citrodora* and *C. winterianus* increased, number of seedlings in pots were decreased and at high application frequency (2x and 3x), *C. winterianus* extract was revealed on par with standard check (Figure 6).

Although number of seedlings per pot treated with essential oil had no variation relative to control, part of the seedlings were damaged and expressed by per cent death. From the present result, per cent death revealed very highly significant ($P < 0.001$) variation (Table 6 and Appendix Table 3). Death percentage of tested weed was high for essential oils extracted from *C. winterianus*, *E. citrodora*, *E. globules*, *P. nigrum* and *R. officinalis* botanicals (Table 6) as compared to the untreated control. Among botanical extracts; essential oil of *C. winterianus* was revealed the highest per cent death on *B. pilosa* seedlings followed by *E. citrodora* on average bases.

Table 5: Effect of botanicals and inert minerals on density of *B. pilosa* under lathhouse conditions at Jimma, Southwestern Ethiopia, in 2011/12

Botanicals	Weed density after treated (per pot)				Efficacy relative to control (%)
	Frequency				
	1x	2x	3x	Mean	
<i>E. citrodora</i>	5.33b ¹	3.00d	0.33 ^e	2.89	85.55
<i>C. winterianus</i>	4.33c	0.00e	0.00e	1.44	92.8
<i>E. globulus</i>	20.00a	20.00a	20.00a	20.00	0.00
<i>T. ammi</i>	20.00a	20.00a	20.00a	20.00	0.00
<i>A. annua</i>	20.00a	20.00a	20.00a	20.00	0.00
<i>P. nigrum</i>	20.00a	20.00a	20.00a	20.00	0.00
<i>R. officinalis</i>	20.00a	20.00a	20.00a	20.00	0.00
<i>R. communis</i>	20.00a	20.00a	20.00a	20.00	0.00
Bole	20.00a	20.00a	20.00a	20.00	0.00
Diatomate	20.00a	20.00a	20.00a	20.00	0.00
H ₂ O ₂	20.00a	20.00a	20.00a	20.00	0.00
Mean	17.24	16.64	16.39	16.76	16.2
Control		20.00a			-
Mamba 360SL		0.00e			100.00
LSD		0.76			-
CV (%)		2.863			-

¹Means followed by different alphabet(s) differ significantly at ($P < 5\%$)

NB:- 1x, 2x and 3x = Sprayed once, twice and trice, respectively

Table 6: Effect of botanicals and inert minerals on percentage death of *B. pilosa* under lathhouse conditions at Jimma, Southwestern Ethiopia, in 2011/12

Botanicals	Scale (ranging from 1 to 9)				Efficacy relative to control (%)
	Frequency				
	1x	2x	3x	Mean	
<i>E. citrodora</i>	3.00g ¹	1.83h	1.3hi	2.04	77.33
<i>C. winterianus</i>	2.67g	1.00i	1.00i	1.56	82.67
<i>E. globulus</i>	7.33c	5.67f	5.83f	6.28	30.22
<i>T. ammi</i>	9.00a	9.00a	8.67ab	8.89	1.22
<i>A. annua</i>	9.00a	9.00a	9.00a	9.00	0.00
<i>P. nigrum</i>	8.33b	7.17cd	7.50c	7.67	14.78
<i>R. officinalis</i>	6.67de	6.17ef	6.00f	6.28	30.22
<i>R. communis</i>	9.00a	8.83ab	8.67ab	8.83	1.89
Bole	9.00a	9.00a	9.00a	9.00	0.00
Diatomate	9.00a	9.00a	9.00a	9.00	0.00
H ₂ O ₂	9.00a	9.00a	9.00a	9.00	0.00
Mean	7.45	6.88	6.82	7.05	21.67
Control		9.00a			-
Mamba 360SL		1.00i			100.00
LSD		0.624			
CV (%)		5.593			

¹Means followed by different alphabet(s) differ significantly at ($P < 5\%$)

NB:- 1x, 2x and 3x = Sprayed once, twice and thrice, respectively, 1 = 100% weed control, 9 = no visible herbicidal effect

4.2.4 Shoot fresh weight

The data on fresh weight (g) of *B. pilosa* plants under different treatment cases was presented in Table 7. Shoot fresh weight as influenced by types of essential oils and with their application frequency was analyzed and the result revealed very highly significant ($P < 0.001$) variations (Appendix Table 4). Application of essential oils from *E. citrodora* and *C. winterianus* as well as standard check resulted significant difference as compared to untreated control. On the other hand, there was no difference for the rest botanicals and

inert minerals compared to control. The highest fresh weight (26.23 g) was obtained as a result of treatment with Bole followed by H₂O₂ (26.11 g) on average base, even though statistically no difference; whereas no seedlings were observed from the standard check followed by *C. winterianus* (2.39 g) on average bases. Similar to other parameters, fresh weight tends to decrease as application frequency increased for *E. citrodora* and *C. winterianus* treatments and the result revealed very highly significant (P<0.001), and the result from *C. winterianus* at high application frequency (2x and 3x) was revealed on par with standard check.

Table 7: Effect of botanicals and inert minerals on shoot fresh weight of *B. pilosa* under lathhouse conditions at Jimma, Southwestern Ethiopia, in 2011/12

Botanicals	Shoot fresh weight (g)				Efficacy relative to control (%)
	Frequency			Mean	
	1x	2x	3x		
<i>E. citrodora</i>	11.53g ¹	6.07h	1.03i	6.21	76.20
<i>C. winterianus</i>	7.17h	0.00i	0.00i	2.39	90.84
<i>E. globulus</i>	25.83a	23.77a-d	21.10def	23.57	9.66
<i>T. ammi</i>	25.87a	25.57ab	25.03abc	25.49	2.30
<i>A. annua</i>	25.2abc	24.8abc	24.73abc	24.91	4.52
<i>P. nigrum</i>	22.57bcd	22.07cde	21.1def	21.91	16.02
<i>R. officinalis</i>	24.2a-d	19.37ef	18.4f	20.66	20.81
<i>R. communis</i>	25.03abc	24.4abc	24.23a-d	24.55	5.90
Bole	26.2a	26.27a	26.23a	26.23	0.54(+)
Diatomate	24.73abc	24.4abc	24.17a-d	24.43	6.36
H ₂ O ₂	26.433a	26.1a	25.8a	26.11	0.08(+)
Mean	22.25	20.26	19.26	20.59	21.08
Control		26.09a			-
Mamba 360SL		0.00i			100.00
LSD		3.144			
CV (%)		9.363			

¹Means followed by different alphabet(s) differ significantly at (P < 5%)

NB:- 1x, 2x and 3x = Sprayed once, twice and trice, respectively, (+) = Stimulated growth

4.2.5 Shoot dry weight

Observation on effects of botanicals application against dry weight (g) recorded is presented in Table 8. There was significant main effect of plant extracts ($P < 0.001$) and application frequency ($P < 0.01$) variations (Appendix Table 4). Among the botanicals, *E. citrodora*, *C. winterianus*, *P. nigrum* and *R. officinalis* recorded significantly lower shoot dry weight (1.11, 0.37, 3.70 and 3.37 gm) respectively, on average base as compared to untreated control. The highest shoot dry weight (4.51 g) was obtained as a result of treatment with Bole on average base followed by untreated control (4.5 g), even though statistically no difference; whereas the seedlings were totally affected as a result of treatment with the standard check followed by *C. winterianus* (0.37 g) on average base. As the application frequency of essential oil of botanicals increased, dry weight of the test weed decreased for these botanicals exhibited significant phytotoxic activity against growth of *B. pilosa*. Hence, the result from *C. winterianus* at 2x and 3x application frequency was revealed on par with standard check.

Table 8: Effect of botanicals and inert minerals on shoot dry weight of *B. pilosa* under lathhouse conditions at Jimma, Southwestern Ethiopia, in 2011/12

Botanicals	Shoot dry weight (g)				Efficacy relative to control (%)
	Frequency				
	1x	2x	3x	Mean	
<i>E. citrodora</i>	1.93h ¹	1.13i	0.27j	1.11	75.33
<i>C. winterianus</i>	1.1i	0.00j	0.00j	0.37	91.78
<i>E. globulus</i>	4.3a-d	4.03a-f	3.8b-f	4.04	10.22
<i>T. ammi</i>	4.57a	4.47ab	4.43ab	4.49	0.22
<i>A. annua</i>	4.27a-e	4.13a-e	4.37abc	4.26	5.33
<i>P. nigrum</i>	3.6efg	3.8b-f	3.7c-g	3.70	17.78
<i>R. officinalis</i>	3.63d-g	3.4fg	3.07g	3.37	25.11
<i>R. communis</i>	4.2a-e	4.07a-f	4.17a-e	4.15	7.78
Bole	4.5a	4.67a	4.37abc	4.51	0.22(+)
Diatomate	4.13a-e	4.1a-e	4.07a-f	4.10	8.89
H ₂ O ₂	4.27a-e	4.07a-f	4.1a-e	4.15	7.78
Mean	3.68	3.44	3.30	3.48	22.67
Control		4.5a			-
Mamba 360SL		0.00j			100.00
LSD		0.688			
CV (%)		12.142			

¹Means followed by different alphabet(s) differ significantly at ($P < 5\%$)

NB:- 1x, 2x and 3x = Sprayed once, twice and trice, respectively, (+) = Stimulated growth

4.2.6 Root fresh weight

The data on root fresh weight (g) of *B. pilosa* weed is presented in Table 9 and measured from randomly selected five normal seedlings that used for measuring root lengths. Root fresh weight as influenced by types of essential oils and their application frequency; hence the result revealed that very highly significant ($P < 0.001$) variations (Table 9 and Appendix Table 4). The highest root fresh weight (6.21 g) was obtained as a result of treatment with Bole on average base followed by untreated control (6.11 g), even though statistically no

difference. The seedlings fresh weight was totally affected as a result of treatment with the standard check followed by *C. winterianus* (0.39 g) on average bases. There was an increasing in inhibitory effect with an increasing in application frequency for few botanical extracts. Evidently, the result from *C. winterianus* at high application frequency (2x and 3x) was revealed on par with standard check and treatments *E. citrodora*, *E. globulus* and *R. officinalis* also application frequent dependant; while insignificantly difference for the rest of botanicals and inert minerals as compared to untreated control.

Table 9: Effect of botanicals and inert minerals on root fresh weight of *B. pilosa* under lathhouse conditions at Jimma, Southwestern Ethiopia, in 2011/12

Botanicals	Root fresh weight (g)				Efficacy relative to control (%)
	Frequency			Mean	
	1x	2x	3x		
<i>E. citrodora</i>	3.83i ¹	1.33j	0.17k	1.78	70.87
<i>C. winterianus</i>	1.17j	0.00k	0.00k	0.39	93.62
<i>E. globulus</i>	5.77a-d	4.67gh	4.37hi	4.94	19.15
<i>T. ammi</i>	6.17a	6.17a	5.94abc	6.09	0.33
<i>A. annua</i>	6.00ab	5.94abc	5.77a-d	5.90	3.44
<i>P. nigrum</i>	5.33b-g	5.2c-g	4.93e-h	5.15	15.71
<i>R. officinalis</i>	5.6a-f	5.07d-h	4.87fgh	5.18	15.22
<i>R. communis</i>	5.83a-d	5.74a-d	5.78a-d	5.78	5.40
Bole	6.05ab	6.22a	6.21a	6.16	0.82(+)
Diatomate	5.98ab	5.75a-d	5.68a-e	5.80	5.07
H ₂ O ₂	5.81a-d	5.80a-d	5.69a-e	5.77	5.56
Mean	5.23	4.72	4.49	4.81	21.28
Control		6.11a			-
Mamba 360SL		0.00k			100.00
LSD		0.776			
CV (%)		9.881			

¹Means followed by different alphabet(s) differ significantly at ($P < 5\%$)

NB:- 1x, 2x and 3x = Sprayed once, twice and trice, respectively, (+) = Stimulated growth

4.2.7 Root dry weight

The data on dry weight (g) of *B. pilosa* weed is presented in Table 10. There was highly significant main effect of plant extracts ($P < 0.001$) and application frequency ($P < 0.01$) variations (Appendix Table 4). Among the botanicals, *E. citrodora* and *C. winterianus* recorded significantly lower root dry weight (0.22 and 0.05 g) respectively, on average base as compared to the control. The highest root dry weight (1.30 g) was obtained as a result of treatment with Bole followed by *T. ammi* (1.28 g), but seedlings were totally affected as a result of treatment with the standard check.

Table 10: Effect of botanicals and inert minerals on root dry weight of *B. pilosa* under lathhouse conditions at Jimma, Southwestern Ethiopia, in 2011/12

Botanicals	Root dry weight (g)				Efficacy relative to control (%)
	Frequency			Mean	
	1x	2x	3x		
<i>E. citrodora</i>	0.46f ¹	0.17g	0.02g	0.22	82.54
<i>C. winterianus</i>	0.14g	0.00g	0.00g	0.05	96.03
<i>E. globulus</i>	1.20a-d	1.04cde	1.02de	1.09	13.49
<i>T. ammi</i>	1.32a	1.25ab	1.28ab	1.28	1.59(+)
<i>A. annua</i>	1.19a-e	1.22abc	1.21a-d	1.21	3.97
<i>P. nigrum</i>	1.13a-e	1.10b-e	1.00e	1.08	14.29
<i>R. officinalis</i>	1.22abc	1.10b-e	1.00e	1.11	11.90
<i>R. communis</i>	1.19a-e	1.22abc	1.24ab	1.22	3.17
Bole	1.31a	1.28ab	1.30a	1.30	3.17(+)
Diatomate	1.21a-d	1.19a-e	1.2a-d	1.20	4.76
H ₂ O ₂	1.23abc	1.20a-d	1.17a-e	1.20	4.76
Mean	1.05	0.98	0.95	0.99	21.43
Control		1.26ab			-
Mamba 360SL		0.00g			100.00
LSD		0.197			
CV (%)		12.174			

¹Means followed by different alphabet(s) differ significantly at ($P < 5\%$)

NB:- 1x, 2x and 3x = Sprayed once, twice and trice, respectively, (+) = Stimulated growth

4.3 Herbicidal effect of tested plants on common weeds of coffee at field trial

4.3.1 Activity against weed density

Similar to the results under lathhouse conditions, essential oils extracted from *E. citrodora* and *C. winterianus* inhibited growth and growth parameters of common weed species in coffee farm at field trial and the results at both conditions (under and out shade) were presented in (Table 11). As application frequency increased, the inhibition of weed density was dramatically increased. Accordingly, fourteen days after treatment, essential oils from *E. citrodora* and *C. winterianus* reduced weed density (Table 11) as compared to the untreated control. Standard check was most toxic to weeds and reduced their density by 100% due to its broad-spectrum nature which was followed by *C. winterianus* at its high application frequency (3x) at both under and out shade conditions on all weed species. Percent weed density reduction over control ranking of top three treatments was standard check > *C. winterianus* > *E. citrodora* on average base. Whereas, *T. ammi* has no substantial effects on weed density compared to control, even at high application frequency.

Unlike to the result obtained under lathhouse conditions, weed species in some plots were not affected totally, but some parts of weeds were damaged especially at the tip of shoots and young leaves and determined by death percentage. The result revealed that very highly interaction effects of botanical species and application frequency of essential oils ($P < 0.001$). Maximum per cent death (100%) was recorded from plots treated with standard check followed by *C. winterianus* and *E. citrodora* at three times application frequency compared to untreated control on average bases.

Table 11: Effect of botanicals on density of weed species under and out shade at Jimma, Southwestern Ethiopia, in 2012

Treatments	Weed density at various conditions											
	Kind of weed											
	Annuals						Perenials					
	Under shade			Out shade			Under shade			Out shade		
Pre-treat	Post-treat	Variation relative to pre-treat (%)	Pre-treat	Post-treat	Variation relative to pre-treat (%)	Pre-treat	Post-treat	Variation relative to pre-treat (%)	Pre-treat	Post-treat	Variation relative to pre-treat (%)	
EC 1x	28.33	29.00	2.35(+)	44.67	46.67	4.48(+)	15.67	20.00	27.66(+)	38.33	40.33	5.22(+)
EC 2x	40.67	32.33	20.49(-)	50.33	35.67	29.13(-)	8.00	7.67	7.99(-)	91.00	88.33	2.93(-)
EC 3x	45.00	29.00	35.56(-)	52.67	37.33	34.82(-)	15.33	10.33	32.61(-)	31.00	22.67	26.87(-)
CW 1x	24.67	25.00	1.35(+)	74.33	77.67	4.49(+)	8.67	11.00	26.92(+)	37.67	41.33	9.72(+)
CW 2x	29.00	16.67	42.53(-)	78.67	64.00	18.65(-)	9.33	5.00	46.43(-)	60.33	56.67	6.07(-)
CW 3x	23.67	8.67	63.38(-)	63.00	40.67	35.44(-)	11.00	5.33	63.11(-)	45.33	36.67	19.10(-)
TA 1x	33.33	38.33	15.00(+)	79.67	85.00	6.69(+)	8.33	11.00	32.01(+)	48.33	55.00	13.80(+)
TA 2x	32.00	37.33	16.67(+)	53.67	60.67	13.04(+)	10.67	14.33	34.37(+)	21.00	25.67	22.24(+)
TA 3x	31.67	37.67	18.95(+)	58.00	68.33	17.81(+)	13.33	17.33	30.00(+)	29.33	33.33	13.64(+)
Sc	42.33	0.00	100.00(-)	86.67	0.00	100.00(-)	11.33	0.00	100.00(-)	40.67	0.00	100.00(-)
Con	35.00	41.00	17.14(+)	53.33	60.67	13.76(+)	13	16.67	28.21(+)	30.00	35.67	18.90(+)

NB:- Con = Control, EC = *E. citrodora*, CW = *C. winterianus*, TA = *T. ammi*, Sc = Mamba 360 SL, 1x, 2x and 3x = Sprayed once, twice and trice, respectively, stimulated (+) and inhibited (-) density of weed species

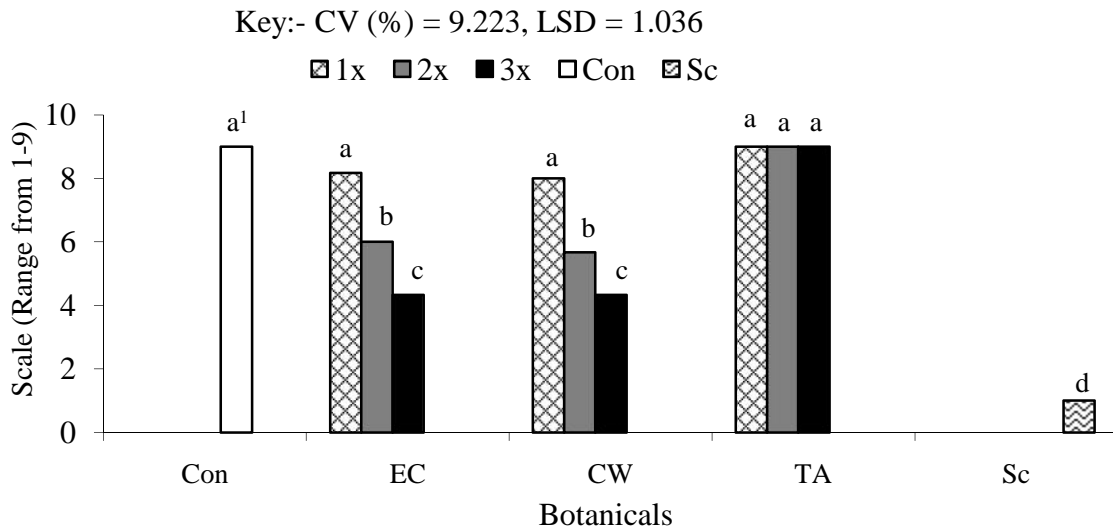


Figure 7: Weed species as influenced by botanicals with different application frequency at Jimma, Southwestern Ethiopia, in 2012

¹Means followed by different alphabet(s) differ significantly at ($P < 5\%$)

NB:- Con = Control, EC = *E. citrodora*, CW = *C. winterianus*, TA = *T. ammi*, Sc = Mamba 360 SL, 1x, 2x and 3x = Sprayed once, twice and thrice, respectively, 1 = 100% weed control, 9 = no visible herbicidal effect

4.3.2 Total biomass

Two weeks after treating, all weeds within a quadrant, both under and out of shade, were separately uprooted and data was recorded and analyzed. For fresh weight of weeds under shade, very highly interaction effects of botanical species and application frequency of essential oils was observed ($P < 0.001$) (Table 12 and Appendix Table 6) and highly significant ($P < 0.01$) variations revealed for fresh weight of weeds out shade. However, there was no significance difference ($P = 0.1154$) and significant ($P < 0.05$) dry weight of weeds under and out shade, respectively. Total biomass measured from each quadrant as influenced by essential oils extracted from *E. citrodora* and *C. winterianus* with their application frequency and sign of injury was observed on all weed species in plots as soon as these oils were sprayed on existed weeds (Figure 8). On the other hand, there was no symptom of injury in plots receive essential oils from *T. ammi* even at high application frequency. Plots treated with standard check revealed sign of injury two days after treating and the symptom proceeding, but the symptoms did not continued for plots treated with oils from *E. citrodora* and *C. winterianus* rather start to recovery. Two weeks after

treating, all weed species within plots treated by standard check were damaged followed by *C. winterianus* at high application frequency (Figure 9). Similar to the results obtained under lathhouse conditions, there was an increase in inhibitory effect with an increase application frequency for essential oils extracted from *E. citrodora* and *C. winterianus*. Evidently, weed populations had a tendency to decrease as application frequency of essential oils extracted from these two plants increased. Weed biomass was totally inhibited (100%) relative to control for plots treated by standard check followed by *C. winterianus* at three times application frequency compared to unsprayed control.

Table 12: Effect of botanicals on total biomass of common weeds of coffee at Jimma, Southwestern Ethiopia, in 2012

Treatments	Fresh weight (g)				Dry weight (g)			
	Under shade	Out shade	Variation relative to control (%)		Under shade	Out shade	Variation relative to control (%)	
			Under shade	Out shade			Under shade	Out shade
EC 1x	265.90 b ¹	322.27ab	18.58	6.23	24.30bc	37.47ab	26.95	10.08
EC 2x	114.67d	215.53c	64.89	37.28	13.57de	31.17b	59.22	25.20
EC 3x	63.03ef	204.7c	80.70	40.44	9.27e	18.27c	72.14	56.16
CW 1x	195.70 c	283.00b	40.07	17.65	20.37cd	37.50ab	38.78	10.00
CW 2x	92.37de	188.4c	71.72	45.18	11.57de	20.73c	65.23	50.24
CW 3x	58.13f	96.57d	82.20	71.90	10.87e	12.53c	67.33	69.92
TA 1x	304.63a	334.77ab	6.72	2.59	31.37ab	40.47ab	5.71	2.88
TA 2x	327.27a	336.17ab	0.21(+)	2.18	35.63a	44.53a	7.11(+)	6.88(+)
TA 3x	322.93a	337.10ab	1.11	1.91	33.73a	42.00 a	1.40(+)	0.80(+)
Control	326.57a	343.67a	-	-	33.267a	41.67a	-	-
Sc	0.00g	0.00e	100.00	100.00	0.00f	0.00 d	100.00	100.00
LSD	33.389	59.645	-	-	8.913	9.798	-	-
CV (%)	8.835	15.049	-	-	27.476	20.085	-	-

¹Means followed by different alphabet(s) within column differ significantly at ($P < 5\%$)

NB:- EC = *E. citrodora*, CW = *C. winterianus*, TA = *T. ammi*, Sc = Mamba 360 SL, 1x, 2x and 3x = Sprayed once, twice and trice, respectively, (+) = Stimulated growth



Control/untreated



C. winterianus before treatment



C. winterianus after treatment



E. citrodora before treatment



E. citrodora after treatment



T. ammi after treatment

Figure 8: Weed species 20-30 minutes after treating as showed sign of injury by essential oils extracted from *C. winterianus* and *E. citrodora* at Jimma, Southwestern Ethiopia, in 2012



Control/untreated



Standard check



T. ammi (3x sprayed)



C. winterianus (3x sprayed)



E. citrodora (3x sprayed)

Figure 9: Weed species as influenced by botanical extracts with different application frequencies at Jimma, Southwestern Ethiopia, in 2012

5. DISCUSSION

From the present finding, use of essential oils extracted from the selected test plants and inert minerals have the potential to retard germination and essential oils extracted from few botanicals inhibited growth of *B. pilosa* as well as on common weed species of coffee. Jefferson and Pennacchio (2003) reported that weed plants that germinated at slower rates are often smaller and less competitive. This could give the opportunity for the crop species to outcompete the weeds (Weiner *et al.*, 1997; Jefferson and Pennacchio, 2003). The plausible reason for inhibition of germination and growth could be due to reduced rate of cell division and elongation of allelochemicals (Javaid and Anjum, 2006). This could be also due to the presence of different allelochemicals within different botanicals which are not compatible with crops (Patil, 2007).

Corresponding with the first hypothesis, there were variations among plant extracts and inert minerals in their potential herbicidal activities in the present study. Accordingly, all essential oils from botanicals and inert minerals greatly inhibited seed germination percentage and rate as well as radicle and plumule lengths of *B. pilosa* as compared to untreated control under laboratory conditions. The inhibitory effects, however, varied among essential oils of plant extracts and inert minerals as well as with respective concentrations. This finding is in line with previous findings that indicated different plants have different allelopathic potential (Adler and Chase, 2007; Price *et al.*, 2008; Mulatu *et al.*, 2011). Olofsdotter (2001) also reported allelopathic potential may vary among genotypes of the same species and Desalegn and Demel (2010) reported the allelopathic effects of *Eucalyptus species* may be species specific. Regarding percent germination reduction over control, ranking of top six plant species were *C. winterianus* > *E. citrodora* > *E. globulus* > *T. ammi* > *R. officinalis* > *P. nigrum* in descending order of their inhibition potential.

Inhibition of essential oils from botanicals and inert minerals on seedling growth under lathhouse was not as much as did on germination and early growth under laboratory conditions and only essential oils from few botanicals inhibited seedlings growth in comparison to untreated. Despite of these variations, the overall ranking of essential oils from botanicals, under lathhouse conditions was quite similar with the ranking based on

the laboratory experiments. Consequently, the growth retardation over control ranking of essential oils from the top four botanical species were *C. winterianus* > *E. citrodora* > *R. officinalis* > *E. globulus* in descending order of their inhibition potential in shoot length (cm) and shoot biomass (g) on average bases. Generally, different plant extracts were found to suppress growth of different weed species (El-Rokiek *et al.* 2006). Singh *et al.* (2005) has been well recognized the allelopathic activity of released compounds of different *Eucalyptus species*. El-Rokiek and El-Nagdi (2011) also reported that germination percentage, seedling root, shoot length and biomass of purslane weed were significantly reduced by leaf extracts of *E. citriodora*. Patil (2007) in similar way depicted that increasing the concentration of aqueous extracts of eucalyptus caused greater decrease in germination, morpho-physical and biochemical parameters of different weed species.

In the case of inert minerals, they showed slight percentage retardation on *B. pilosa* seed and low inhibition effect on early growth as compared to essential oils obtained from tested plants in Petri dishes. Furthermore, these minerals and essential oils from few botanicals show slightly stimulated seedlings growth and biomass over control in pot culture though statistically similar. Accordingly, shoot length increased over control ranking of top three treatments were *R. communis* > H₂O₂ > Bole in descending order of their stimulant potential in pot culture for shoot length (cm) on average bases. In case of total biomass (shoot and root biomass), only Bole slightly stimulated over control and among all treatments. Jabeen and Ahmed (2009) reviewed that plant may exhibit inhibitory or rarely stimulatory effects on germination and growth of other plants in the immediate vicinity.

From the present study at both experimental conditions, essential oils from most of the selected test plants and inert minerals showed percent germination reduction over control in Petri dishes, whereas essential oils from few botanicals revealed growth and growth parameter reduction over control in pot culture. This finding is in line with the previous reports that demonstrated about the allelopathic effects of *C. citratus* to have toxicant, repellent and fumigant activities against pests (Odeyemi, 1993; Zaridah *et al.*, 2003; Saljoqi *et al.*, 2006). *E. citrodora* had inhibitory effect on the seed germination of different crops (Singh *et al.*, 1992; Zhang *et al.*, 2002; Singh *et al.*, 2005). Ramezani *et al.* (2002) again reported that volatile oils from *E. citriodora* and its major constituent possess fungitoxic activities for the bio-management of plant diseases. *E. globules* had allelopathic

effect on different crop seeds germination and growth parameters (Muhammad *et al.*, 2007; Muhammad *et al.*, 2008; Muhammad *et al.*, 2009) and adversely affected plant population of cotton (Sahadeva *et al.*, 2006). The essential oils of *E. globules* were also reported to have contact toxicity and highly effective against different insects which damage cereal crops at storage conditions (Srivastava *et al.*, 1988; Araya, 2007). Paudel and Gupta (2008) reported essential oils from *C. citratus* and *E. citrodora* at their different concentrations have adverse effects on the seed germination and seedling length of *Parthenium* weed at varying degree. Katiyar *et al.* (2011) reported *C. winterianus* was known for its natural insect repellent properties.

Koul *et al.* (2008) and Mohan *et al.* (2011) in similar way reported the pest control properties of *C. citratus*, *R. officinalis*, *E. globules*, *T. ammi* and *P. nigrum*. Pattnaik *et al.*, (2012) also depicted that inhibitions of fungal growth were observed by *P. nigrum* for *Septoria lycopersici*. *A. annum* has been also shown to be an effective non-selective herbicide (Duke *et al.*, 1987) and had inhibitory effects and significantly reduced weed emergence and weed dry weight (Delabays, u.d). Esayas *et al.* (2012) depicted the efficacy of essential oils from *C. nardus*, *C. martini* and *R. officinalis* against major coffee insect pests. Tounou *et al.* (2011) reported that castor bean plant extracts and oil could be toxic to larvae of *P. xylostella* through contact and ingestion. Buss and Park-Brown (2009) documented Diatomate may control slugs, millipedes and sow bugs, as well as soft-bodied insects like aphids. So far only few reports are available on the allelopathic effects of Bole, and H₂O₂.

Application of essential oils extracted from all botanicals, inert minerals and H₂O₂ treatments showed from moderately to high level of dose-dependent on *B. pilosa* seed germination inhibitory effects. But only application of extracts from *E. citrodora*, *C. winterianus*, *E. globulus*, *T. ammi*, *R. communis* and *A. annua* at 0.06 and 0.09% (v/v) concentration showed more than 50% weed germination inhibitor levels, whereas; extracts from *P. nigrum*, *R. officinalis* and Diatomate revealed more than 50% inhibition only when applied at concentration of 0.09% (v/v). Speed of germination and early growth of *B. pilosa* weed were also reduced as concentration of essential oils and inert minerals increased. This is in agreement with second hypothesis and several authors, (Muhammad *et al.*, 2008; Muhammad *et al.*, 2009; Desalegn and Demel, 2010) reported allelopathic effects of *Eucalyptus species* was concentration dependent and similarly (El-Darier, 2002;

Mulatu *et al.*, 2005; Araya, 2007; Mulatu *et al.*, 2011) reported the inhibition of plant extracts were concentration dependant. Jadhar and Gayanar (1992) and Bora *et al.* (1999) also reported that the inhibitory effect of leaf extracts of *A. auriculiformis* on germination, plumule and radicle length of some agricultural crops was proportional to the concentration of the extract. Desalegn and Demel (2010) reported the allelopathic effect of *E. globules* was concentration dependant and the mechanism for the inhibition could be attributed to osmotic effects of the extract.

In the present study, few plant extracts reduced the growth and total biomass of *B. pilosa* seedlings over control in pot culture under lathhouse conditions and the inhibitory effects, however, varied among essential oils of plant extracts as well as with respective application frequency for the same plant. Consequently, at lower application frequency (1x), comparatively less reduction in fresh weights recorded than at higher application frequency (3x). From the present finding, *B. pilosa* seedlings showed signs of injury within 20-30 minutes of treatment, and maximum injury occurred within 24 hour of application of the essential oils from *C. winterianus* and *E. citrodora* in pot experiment which is in line with the previous finding (Tworkoski, 2002). El-Rokiek and El-Nagdi (2011) reported that seedling root and shoot length of purslane weed were significantly reduced by leaf extracts of *E. citriodora*, as concentration increased. Three times application frequencies of essential oils from *C. winterianus* and *E. citrodora* showed total loss of *B. pilosa* seedlings on par with standard check. Essential oils from *R. officinalis* and *E. globules* also showed signs of injury at the tip of shoots and young leaves, but recovered quickly. This is congruent with the previous finding (Lanini, 2010).

The probable reason for inhibition of seedlings' growth and reduction of total biomass could be due to the residual effect of first application which facilitated or conditioned the inhibition of next application. Accordingly, essential oils from the top two botanicals; *C. winterianus* and *E. citrodora* provided greater reduction in seedling growth and total biomass as compared to untreated control. This finding is in agreement with the third hypothesis and Buss and Park-Brown (2009) who reported that more frequent applications may be necessary, since; essential oils from botanicals degrade rapidly in sunlight, air, and moisture, and by detoxification enzymes. Sahadeva *et al.* (2006) and Muhammad *et al.* (2008) stated aqueous extracts of different concentrations *Eucalyptus species* significantly reduced the fresh and dry weight of wheat seedlings over control. Turk *et al.* (2005) also

reported aqueous extracts of *B. nigra* significantly inhibited radish seedling biomass. El-Rokiek and El-Nagdi (2011) also disclosed that reduction in biomass of purslane weed was recorded by foliar treatment of aqueous extracts of *E. citrodora* 81 days after sowing, in comparison to untreated control.

Compared to laboratory results, the present study clearly showed that less inhibitory effects by plant extracts and inert minerals were observed on seedling growth under lathhouse conditions. From the present study essential oils from all botanicals and inert minerals provided a strong reduction on germination percentage and rate as well as plumule and radicle length under laboratory conditions. But the inhibitory effects on seedlings growth and other parameters were observed only for essential oils extracted from *C. winterianus*, *E. citrodora*, *R. officinalis* and *E. globulus* under lathhouse conditions. This is in line with the findings of previous workers (Morgan and Overholt, 2005; Mulatu *et al.*, 2011). This could be explained by the complex reactions taking place in the soil. Allelochemicals are subjected to various biotic and abiotic processes that reduce their persistence, concentrations, availability and biological activities after they are added into the soil (Makino *et al.*, 1996; Blum *et al.*, 1999; Okumura *et al.*, 1999).

Based on results of the two preliminary experiments, field trial was carried out with essential oils extracted from *C. winterianus*, *E. citrodora* and *T. ammi* on existed weed species in coffee farm at JARC in the same concentration and application frequency with that of lathhouse experiment. The result revealed that highly significant at high application frequencies by essential oils from the first two candidate botanicals whereas there was no difference for oils from *T. ammi* related to untreated control for all growth parameters. In contrast to the results obtained under lathhouse, the inhibition of essential oils from *C. winterianus* and *E. citrodora* were not as effective as standard check that means no total loss even at high application frequencies at field trial. The possible reason for low inhibition of growth could be due to high volatile nature of essential oils which is also confirmed with other works that oil of botanicals degrade rapidly in sunlight. This also in line with the present finding that, variation of weeds treated with essential oils relative to control were high for weeds under shade as compared to out shade conditions.

The effect of essential oils extracted from different plants on germination and growth at various conditions showed inconsistency; however, oils from *C. winterianus* and *E. citrodora* provided greater reduction in germination percentage, plumule and radicle elongation as well as common weeds of coffee growth as compared to untreated control. Therefore, the herbicidal effect of essential oils extracted from *C. winterianus* and *E. citrodora* on growth and growth parameters of common weed species of coffee at different experimental conditions showed consistency in inhibition potential. To date, reports are not available which deal with the herbicidal potential of these plants against common weed species of coffee. But there are reports (El-Rokiek and El-Nagdi, 2011) pertaining to herbicidal potential of *E. citrodora* on purslane and Muhammad *et al.* (2008) observed aqueous extracts of different concentrations *Eucalyptus species* significantly reduced the fresh and dry weight of wheat seedlings over control.

6. SUMMARY AND CONCLUSION

Weeds are one of the most important constraints that limit crop production worldwide in general and in organic farm in particular, since, organic farms do not permit to use synthetic herbicides. On the other hand, weed control without chemicals is a great challenge. Mechanical/physical, cultural, biological weed control, etc. can be used in organic farm, but these techniques can be costly and may fail to control weeds adequately. Using natural product herbicides are an alternative for weed control in organic farm. Essential oils are extracted from plants and thus may be useful as naturally originated herbicides for organic farming systems in Ethiopian coffee production systems.

The present study was conducted to explore botanical herbicides from local plants against common weed species with emphasis on germination and growth of *B. pilosa* in organic coffee system in Ethiopia. A series of experiments were conducted with essential oils extracted from some spices and/or herbs and inert minerals. The results of different experiments confirmed that there were consistencies herbicidal effects due to use of tested botanicals, which varied from laboratory to field experimental conditions. Among the botanicals were evaluated, essential oils from *C. winterianus*, *E. citrodora*, *E. globules*, *T. ammi*, *R. officinalis* and *P. nigrum* were the best six botanicals in terms of consistency of suppressing seed germination. Germination was faster for seeds treated with only distilled water followed by H₂O₂ on average base, whereas the lowest rate of seed germination was recorded from seeds treated with essential oils from *C. winterianus*. Plumule and radicle length inhibition was not as severe as germination inhibition. Accordingly, the tallest plumule growth and radicle length were obtained as a result of treatment with distilled water followed by H₂O₂ and essential oils from *R. officinalis*, respectively, whereas; the shortest lengths for both were observed from the standard check followed by essential oils from *C. winterianus*.

In contrast to the results obtained under laboratory, only essential oils from few botanicals had inhibitory effects on *B. pilosa* seedlings, whereas; essential oils from other botanicals and all inert minerals didn't rather stimulated growth and growth parameters under lathhouse conditions. Regardless of these differences, oils extracted from *C. winterianus*, *E. citrodora*, *R. officinalis* and *E. globulus* revealed persistently inhibitory effects at both

conditions. Under laboratory conditions, there was an increase in inhibition effect on both germination and early growth with an increase in extract concentration. Similarly, under lathhouse conditions, inhibition was high as application frequency increased in most of the treatments.

Essential oils from *E. citrodora*, *C. winterianus* and *T. ammi* were also checked for their potential as herbicides against common weed species of coffee under field conditions and the results revealed that their effect was highly significant for the first two botanicals and no difference for essential oils from *T. ammi* related to untreated control for all growth parameters. The results from the present finding suggest that essential oils from various botanicals and inert minerals types, level of concentration, application frequency and their interaction have sound and promising impact on common weed species of coffee especially on germination and early growth of *B. pilosa*. The degree of inhibition varied, according to the type of essential oils from botanicals as well as inert minerals with their concentration and application frequency. Essential oils extracted from *E. citrodora* and *C. winterianus* revealed consistent inhibitory effects on growth and growth parameters of weed species at various experimental conditions. The effect of essential oils from the two botanicals was concentration and application frequency dependent.

Generally, essential oils from *E. citrodora* and *C. winterianus* combined with high concentration level significantly decreased germination percentage and rate; early growth of *B. pilosa*. Similarly essential oils from these two botanicals highly affected the growth and growth parameters of *B. pilosa* seedlings and common weeds of coffee at three times application frequency under lathhouse and field conditions, respectively. But their effects were not genetically compared as that of application of the standard check. Therefore, it can be concluded from this study that essential oils from *E. citrodora* and *C. winterianus* at high concentration and three times application frequency have the potential to retard germination and growth weed species in coffee farm.

7. RECOMMENDATIONS FOR FUTURE WORKS

From the present study there are some clues about potential efficacy of essential oils from botanicals and inert minerals as a means to control common weeds of coffee especially the *B. pilosa* in respect of seed germination and early growth. However, further research would be imperative to come up with sound recommendation of these essential oils and inert minerals for practical use as a component of integrated weed management methods. Consequently, it is important to know the active ingredient inherited from the source plant, mode of action on the receptor plant, rate as well as time of application, and mechanisms of defense by the receptor plant. Therefore, the following points are suggested for further research to broaden our understanding on the mechanisms involved and thereby to optimize the weed suppressive effects of botanical herbicides under field conditions especially to promote organic farming systems.

To increase our understanding, the following aspects could be considered for further investigations:

- ✿ Standardization of concentration and frequency of application
- ✿ Mode of action and impact on other target crops as well as on biotic factors
- ✿ Rate and time of application for essential oils
- ✿ Economic feasibility studies to use essential oils from botanicals as alternative or complementary weed control means
- ✿ Mechanism and site of action of allelochemicals from the potential botanicals
- ✿ Characterization of the active ingredient(s) and to establish the mechanism of herbicidal action with other essential oils

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9. APPENDICIES

Appendix 1: Mean squares and P values

Appendix Table 1: Means square and P values of *B. pilosa* germination percentage and rate as well as early growth as influenced by botanicals and inert minerals under laboratory at Jimma, Southwestern Ethiopia

Source of Variation	DF	Germination (%)		Germination rate (seeds per day)		Plumule length (cm)		Radicle length (cm)	
		MS	P	MS	P	MS	P	MS	P
Replication	2	0.0648	0.052	0.0085	0.2479	0.1377	0.0366	0.0351	0.7579
Ex	10	11.6036	<.0001	7.0844	<.0001	1.3974	<.0001	5.1916	<.0001
Conc	2	61.4456	<.0001	20.6076	<.0001	18.463	<.0001	12.806	<.0001
Ex*Conc	20	2.9292	<.0001	0.5574	<.0001	0.4564	<.0001	1.2247	<.0001
Error	64	0.0209		0.0059		0.0395		0.126	
Total	98	-		-		-		-	
LSD		0.236		0.126		0.324		0.579	
CV (%)		2.108		2.559		9.556		9.332	

DF = Degree of Freedom MS = Mean Square P = P value, Ex = Extraction, Conc = Concentration

Appendix Table 2: Means square and P values of *B. pilosa* growth as influenced by botanicals and inert minerals under lathhouse at Jimma, Southwestern Ethiopia

Source of Variation	DF	Shoot length (cm)		Root length (cm)	
		MS	P	MS	P
Block	2	0.041	0.0648	0.7877	0.1172
Ex	10	7.1348	<.0001	20.5825	<.0001
Freq	2	2.2023	<.0001	5.1579	<.0001
Ex*Freq	20	0.7418	<.0001	1.6201	<.0001
Error	64	0.0144		0.3552	
Total	98	-		-	
LSD		0.1954		0.9721	
CV (%)		3.1833		10.2236	

DF = Degree of Freedom MS = Mean Square, P = P value, Ex= Extraction, Freq= Frequency of application

Appendix Table 3: Means square and P values of weed density and death percentage of *B. pilosa* as influenced by botanicals and inert minerals under lathhouse at Jimma, Southwestern Ethiopia

Source of Variation	DF	Weed Density (per pot)		% Death	
		MS	P	MS	P
Block	2	0.6364	0.0705	0.1086	0.5011
Ex	10	469.3071	<.0001	70.6303	<.0001
Freq	2	6.3030	<.0001	4.0707	<.0001
Ex*Freq	20	3.1253	<.0001	0.5068	0.0002
Error	64	0.2301		0.1555	
Total	98	-		-	
LSD		0.7601		0.6236	
CV (%)		2.8626		5.5923	

DF= Degree of Freedom, MS= Mean Square, P= P value, Ex= Extraction, Freq= Frequency of application

Appendix Table 4: Means square and P values of *B. pilosa* biomass as influenced by botanicals under lathhouse at Jimma, Southwestern Ethiopia

Source of Variation	DF	Fresh weight (g)				Dry weight (g)			
		Shoot		Root		Shoot		Root	
		MS	P	MS	P	MS	P	MS	P
Block	2	14.0948	0.0277	0.4664	0.1355	0.0876	0.6139	0.0304	0.1342
Ex	10	615.7268	<.0001	32.8639	<.0001	17.6706	<.0001	1.7038	<.0001
Freq	2	76.7003	<.0001	4.7276	<.0001	1.2112	0.0021	0.0979	0.0023
Ex*Freq	20	10.6732	0.0007	0.9562	<.0001	0.272	0.1029	0.0161	0.3755
Error	64	3.716		0.2261		0.1781		0.0146	
Total	98	-		-		-		-	
LSD		3.144		0.7757		0.6884		0.1974	
CV (%)		9.3632		9.8809		12.1416		12.1739	

DF=Degree of Freedom, MS=Mean Square, P=P value, Ex= Extraction, Freq=Frequency of application

Appendix Table 5: Means square and P values of death percentage of common weeds of coffee as influenced by botanicals under field at Jimma, Southwestern Ethiopia

Source of variation	DF	% Death	
		MS ²	P ³
Block	2	1.694	0.0390
Ex	2	25.583	<.0001
Freq	2	14.250	<.0001
Ex*Freq	4	3.583	0.0007
Error	16	0.4234	
Total	26	-	-
LSD		1.036	
CV (%)		9.223	

DF=Degree of Freedom, MS=Mean Square, P=P value, Ex= Extraction, Freq=Frequency of application

Appendix Table 6: Means square and P values of total biomass of common weeds of coffee as influenced by botanicals under field at Jimma, Southwestern Ethiopia

Source of Variation	DF	Fresh weight (g)				Dry weight (g)			
		Under shade		Out shade		Under shade		Out shade	
		MS	P	MS	P	MS	P	MS	P
Block	2	610.367	0.157	402.618	0.768	8.227	0.787	21.160	0.6018
Ex	2	106880.135	<.0001	49104.751	<.0001	1041.335	<.0001	838.413	0.0008
Freq	2	27616.499	<.0001	23554.298	0.0002	128.674	0.0447	456.189	0.0009
Ex*Freq	4	10774.908	<.0001	7592.719	0.0079	74.486	0.1154	164.841	0.0181
Error	16	293.350		1502.857		33.882		40.359	
Total	26	-		-		-		-	
LSD		33.389		59.645		8.928		9.798	
CV (%)		8.835		15.049		27.476		20.085	

DF=Degree of Freedom, MS=Mean Square, P=P value, Ex= Extraction, Freq=Frequency of application

Appendix Table 7: Simple correlation on germination and early growth of *B. pilosa* under laboratory at Jimma, Southwestern Ethiopia

Variables	GP	GR	PL	RL
GP	1.00	0.829***	0.783***	0.864***
GR		1.00	0.789***	0.639***
PL			1.00	0.629***
RL				1.00

***= very highly significant difference at 5% probability levels, GP= Germination Percentage, GR= Germination Rate, PL= Plumule Length, RL= Radicle Length

Appendix Table 8: Simple correlation on growth parameters of *B. pilosa* seedlings under lathhouse at Jimma, Southwestern Ethiopia

Variables	SHL	RL	SHFW	RFW	SHDW	RDW	WD	DP
SHL	1.00	0.915***	0.918***	0.916***	0.906***	0.865***	0.888***	0.881***
RL		1.00	0.870***	0.891***	0.860***	0.847***	0.874***	0.823***
SHFW			1.00	0.957***	0.959***	0.933***	0.947***	0.935***
RTFW				1.00	0.953***	0.941***	0.930***	0.929***
SHDW					1.00	0.934***	0.935***	0.927***
RDW						1.00	0.951***	0.926***
WD							1.00	0.914***
DP								1.00

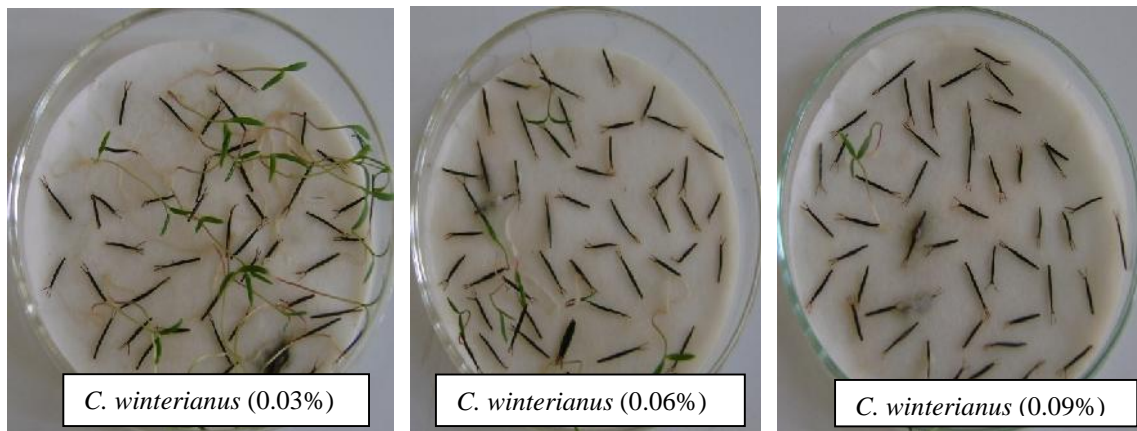
***= very highly significant difference at 5% probability levels, SHL= Shoot Length, RL= Root Length, SHFW= Shoot Fresh Weight, RFW= Root Fresh Weight, SHDW= Shoot Dry Weight RDW= Root Dry Weight, WD= Weed Density, DP= Death Percentage

Appendix Table 9: Simple correlation on growth parameters of common weeds of coffee under field at Jimma, Southwestern Ethiopia, in 2012

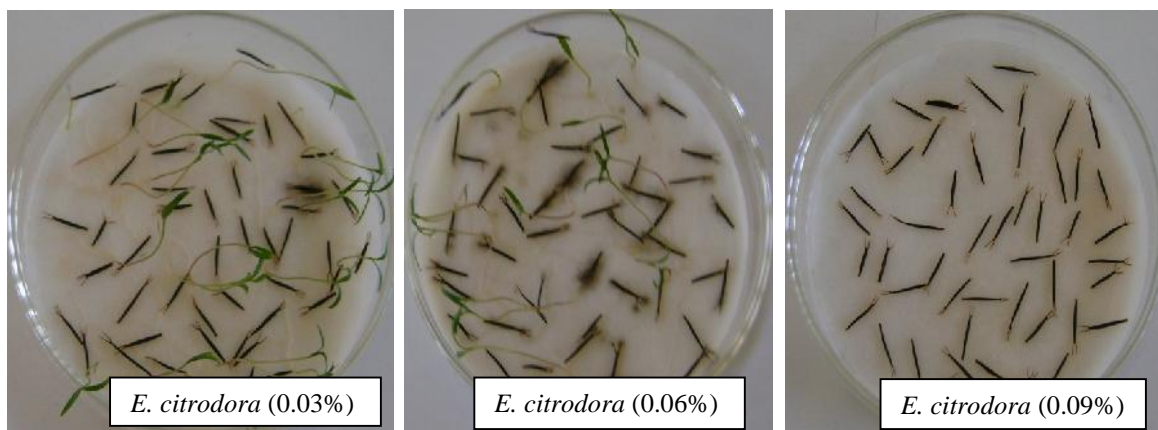
Variables	AWDUSH	AWDOSH	PWDUSH	PWDOSH	FWUSH	FWOSH	DWUSH	DWOSH	DP
AWDUSH	1.00	0.769***	0.899***	0.777***	-0.898***	-0.899***	-0.795***	-0.880***	-0.867***
AWDOSH		1.00	0.894***	0.817***	-0.813***	-0.781***	-0.756***	-0.732***	-0.923***
PWDUSH			1.00	0.833***	-0.866***	-0.890***	-0.763***	-0.856***	-0.934***
PWDOSH				1.00	-0.749***	-0.740***	-0.648***	-0.660***	-0.878***
FWUSH					1.00	0.859***	0.904***	0.844***	0.904***
FWOSH						1.00	0.811***	0.863***	0.830***
DWUSH							1.00	0.841***	0.824***
DWOSH								1.00	0.806***
DP									1.00

***= very highly significant difference at 5% probability levels, AWDUSH= Annual Weed Density under Shade, AWDOSH= Annual Weed Density out Shade, PWDUSH= Perennial Weed Density under Shade, PWDOSH= Perennial Weed Density out Shade, FWUSH= Fresh Weight under Shade, FWOSH= Fresh Weight out Shade, DWUSH = Dry Weight under Shade, DWOSH = Dry Weight out Shade, DP= Death Percentage

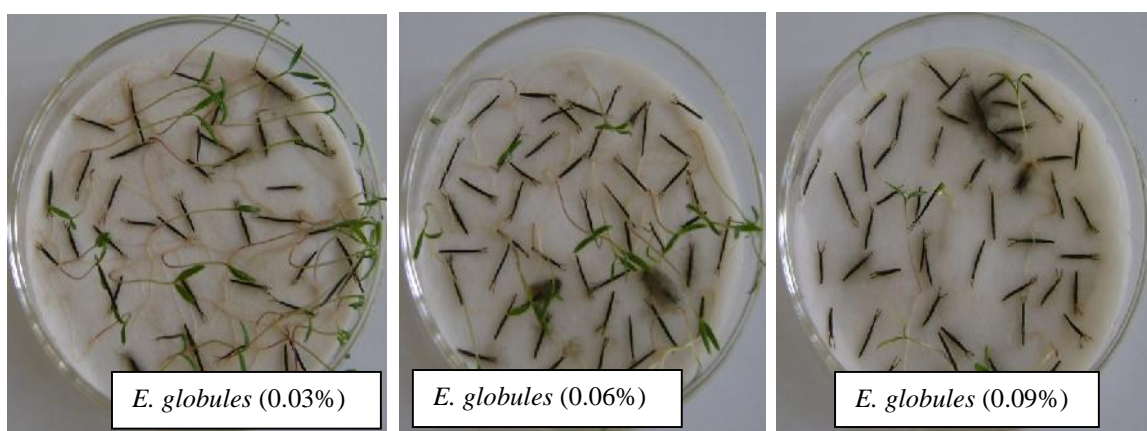
Appendix 2: Figures of the Experimental at various conditions and Equipments



Appendix Figure 1: *B. pilosa* seed germination and early growth as influenced by *C. winterianus* under laboratory at Jimma, Southwestern Ethiopia



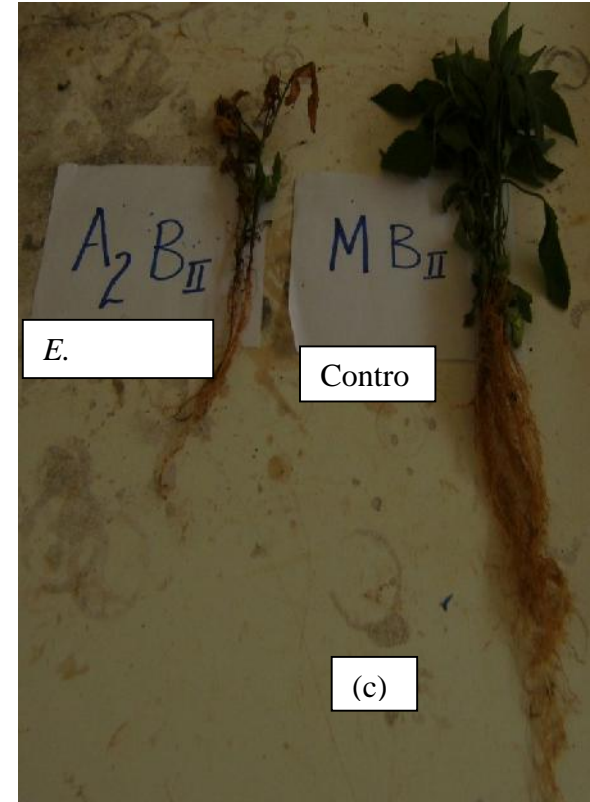
Appendix Figure 2: *B. pilosa* seed germination and early growth as influenced by *E. citrodora* under laboratory at Jimma, Southwestern Ethiopia



Appendix Figure 3: *B. pilosa* seed germination and early growth as influenced by *E. globules* under laboratory at Jimma, Southwestern Ethiopia



Appendix Figure 4: Preparation of essential oils and inert minerals for application (a), sprayer (b), flasks (c), under lathhouse at Jimma, Southwestern Ethiopia



Appendix Figure 5: Roots of *B. pilosa* seedlings taken before washing (a), after washing (b), comparison of *E. citrodora* with distilled water (c), fourteen days after treating under lathhouse at Jimma, Southwestern Ethiopia