

Efficacy of Natural Oils from Eucalyptus Species against Cochineal Insect

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Abstract - Due to the immense important of it has, cochineal insect was introduced to Tigray Regional State of Ethiopia. However, due to wild use of this insect, the cactus pear which is the integral part of economy of the people is being destroyed. Let alone to get additional benefit which was assumed during the introduction of this insect, the enormous benefits of this fruit which have been for many years is being lost. Currently, the people of Tigray, especially, the northern part lost their whole cactus pear. What makes it more sever is that, the insect is rapidly expanding to the rest of the area where cactus pear and the people are highly interconnected. This study was aimed to evaluate the efficacy of natural oils from Eucalyptus against cochineal insect. The essential oil used here was obtained by hydro distillation from fresh *Eucalyptus camaldulensis* leaf. The oil recorded high insecticidal activity both in laboratory and spray test. Death time for young male and female was 38 ± 10 , 80 ± 15 min respectively. Corresponding sex adults die at 72 ± 13 and 86 ± 14 at higher concentration ($100\mu\text{l}/\text{air}$). In three days spray of $600\mu\text{l}/\text{air}$ oil all insects of all ages were eliminated.

Keywords - *Opuntia ficus indica*, *Eucalyptus camaldulensis*, Hydro Distillation, Essential Oil, Phytochemical Screening, Insecticidal

1. Introduction

1.1. Background and Justification

Cactus is widely distributed and used in the region than any other places in Ethiopia. Specifically, in Adigrat town and surrounding areas as it is owned by individual farmers. The use of the cladode as fodder for cattle has similar beginning to the arrival of cactus by missionaries [1, 2, 3]. The fruits themselves dated long since the recognized as favorite fruits. Recently cactus salads and juices are familiar to the area. With the current global environmental change cactus is promising due to its drought tolerant properties [4, 5]. Food security is one of the major plans of the country in its twenty years transformation plan and the continents major goal [6, 7]. However, cactus is been gradually destroyed. With its potential recognized the loss is severe. Because;

The farmers are still preparing fodder even from the infected ones. Most pests produce toxic substances against their hosts while the white powder produced by cochineal insect is acid (carmine acid). May be this is the only chance they have than to see their cattle's dying from starvation. The infected ones may lead them to death in cumulative effect. To lose the cattle's is one problem; to lose the fodder is another major long lasting problem need to be addressed immediately.

The use of cactus cladodes to the farmers as fodder for their cattle, sheep, camel and goats for one Solid year and as their seasonal favorite fruit for 3-4 months is far more useful than to the die industry which uses carmic acid produced from the insects (even though there is no such industry in over all the country). Life of their cattle's can never be an option over use of the insect in giving die. The source of income by selling the fruits has covered house expenses of many poor farmers and educational expenses of many poor students especially girls.

No report appear on the literature with remedy to the infected plants. Some chemical pests are suggested however, much time is needed to study the side effect and cost analysis.

Concerned action has not done even as immediate solution for the problem. The destruction of the cladodes is one thing but the effect of the carmic over the animals who probably consumed the infected cladodes is another thing that should be considered seriously.

There is no time to stay before even the infection become unmanageable and widespread in over all the country.

The eucalyptus oils we employed here have use but no harm to the plant as they are plant derived, eco-friendly and volatile.

In the eve of the day to recognize the potential use of cactus, this is severe economic lose that needs the attention of any stakeholder in the field and the region in general.

1.2. Eucalyptus

1.2.1. Chemistry

The family of Eucalyptus (Myrtaceous) plants are source of biologically active terpenoids and polyphenols, including flavonoids, phloroglucinol derivatives, and tannins [8]. More than 300 species of this Eucalyptus genus contain volatile oils in their leaves [9, 10]. Numerous studies done on Eucalyptus species report the isolation of various phytoconstituents.

Phytochemical constituents such as steroid and fatty acids (wood) [11], tannins, poly phenolics, glycosides, terpenes, alkaloids, flavonoids, Flavonoids, Alkaloids, Saponins, Lignin's, Vitamin C, Tannin, Fatty Acids , Phenolics, Triterpenoid, Flavones, Anthocyanin, Anthraquinone, Steroids, Coumarins, Cardiac glycosides on different solvent extract of the bark [12]. Leaf essential oils on the other hand found out to contain flavonoids tannins, alkaloids, glycosides, terpenoids, and steroids [13]. Another study also showed the presence of Alkaloids, Tannins, Flavonoids and Phenolics in methanolic extract of the leaf [14].

GC-MS screening of essential oils of eight eucalyptus species showed that the main components were 1,8-cineole followed by cryptone , α -pinene, p-cymene, α -terpineol, trans pinaocarveol, phellandral, cuminal, globulol, limonene, aromadendrene, saphulenol and terpinene-4-ol [10]. Similar research done on *Eucalyptus globulus* also reported the presence of ten compounds. Those are Stigmasterol, Ursolic acid, Rhodomyrtone, Eucalyptone G, 4', 5, 7-Trimethoxykaempferol, Naringenin, Genistein, Catechin, Epicatechin, Octyl- β -D-glucopyranoside [8]. The same assay on Eucalyptus globulus essential oils reported the presence 1.8 cineole (85.8%), α -pinene (7.2%), and β -myrcene (1.5%) being the main components. Other compounds identified in the oil were β -pinene, limonene, α -phellandrene, γ terpinene, linalool, pinocarveol, terpinen-4-ol, and α -terpineol also Alpha-pinene, Beta-pinene, Limonene, Eucalyptol (1,8-cineole), p-cymene, Pinocarvone, Aromadendren, Trans-pinocarveol, Alpha-terpineol, Terpinyl-acetate and Viridiflor was revealed to present as to another research work on *Eucalyptus globulus* [15]. Even though more studies are done on *Eucalyptus globulus*, as far as they are in the same genera there will not be significant difference in their chemical composition with *Eucalyptus camaldulensis*. For instance, in phytochemical composition one study proved to have Tannins, Saponins, Glycosides, Steroids, Anthraquinones in over all the plant parts [16]. Essential oils of *E. camaldulensis* leaf from one study tend to contain 37 compounds With eucalyptol (29.2%), α -phrllanderene (17.43%), α -pinene (7.1%), aromadenderene (allo) (5.75%), terpineol-4-ol(4.92%) the major components. Another research work from different area also isolated p-Cymene, α -Terpinene, 1,8-Cineole, Terpinen-4-ol, α -Pinene, α -Terpineol from the leaf of oils of the same plant.¹⁷ In a search for cytotoxic effects one investigation also find out α -Pinene, α -Phellandrene, α -Terpinene, o-Cymene, Limonene, 1,8-Cineole, γ -Terpinene, Terpinolene, Linalool, Terpinen-4-ol, α -Terpineol, Piperitone, Globulol, γ -Eudesmol, δ -Cadinol in essential oil from leaves, stems and immature flowers of *Eucalyptus camaldulensis* Dehnh [18]. This and other compounds were also reported from research work on antimicrobial value of that plant essential oil [19].

1.2.2. Biological Activity

Eucalyptus globulus: As intensive studies have done in this plant findings from different research reveal to have curing capacity to many more alignments.

Traditionally it is used as remedy for wounds, tumor, dyspepsia, malaria, abscess, arthritis, inflammation, asthma, boils, bronchitis, burns, cancer, cold, cough, diabetes, diphtheria, dysentery, fever, miasma, sore throat, spasms, tuberculosis, vaginitis, and worms [15, 20, 21, 22].

Experimentally (in vitro) it has proved to have antimicrobial [12, 13, 15, 21, 23], miticide [23], Antioxidant [24] antiperiodic, antiphlogistic, antiseptic, astringent, deodorant, anthelmintic, diaphoretic, expectorant, inhalant, insect repellent [25, 26], pesticide [27] rubefacient, sedative yet stimulant, suppurative, and vermifuge efficacy [11].

Eucalyptus camaldulensis: also tend to possesses many biological activities such as;

Traditionally to treat sore throat, anesthetic, wound, dysentery, astringent, antiseptic, diarrhea, cold, hemorrhage, cough, fever and wide range of ailments(In the Igala societies it is known by the name "Ogwu iba" meaning fever remedy), laryngalgia, laryngitis, spasm, trachagia vermifuge, gastrointestinal symptoms, arrest bleeding, open wounds and cuts, of aches and pains in muscles, joints and even tooth, bladder infections, enteric infections (constipations and other stomach problems), asthma, oral thrush, boils, sores, asthma, bronchitis, eczema and athletes foot [28, 29].

Many research works (in vitro) also show its Antifungal [17], cytotoxic [18, 30], antimicrobial, [9, 28, 29, 31, 32, 33] anti-dermatophyte [9] activity.

1.3. Cactus (*Opuntia ficus indica*)

1.3.1. Overview

The cactus plant belongs to the subfamily Opuntioideae and comprises more than 200 species worldwide. In Mexico 114 species exist, cacti. Based in their wide geographic distribution and the great diversity of habitats they occupy the cactus pear are among the most versatile cacti in the family. Being a source of fruit ("tunas") and vegetables ("nopalitos") for human consumption, as well as fodder for cattle and other animals during the dry seasons in South America especially Mexico, Spain and in arid and semiarid areas around the world [34].

1.3.2. Chemical Composition

As in many review researches on cactus pear fruits, it was found out to have high nutritional value due to their high amounts of sugar and minerals. Production of juices, marmalades, gels, dehydrated foods, jams and jellies, natural sweeteners, wine and other alcoholic beverages, candies, and canned and frozen fruitand other products are possible from the cladodes [35, 36, 47].

Prickly pear (*opuntia ficus indica*) seeds flour contains significant amount of protein, fat, fiber, carbohydrate, moisture and many essential amino acids such as histidine, isoleucine, leucine, lysine, methionine, phenylalanine [36, 38]. Study done at five developmental stage based on length of cactus cladodes find out the presence of lipid, vitamin-C, protein, fat, fiber, ash, carbohydrate, moisture and carotene [39]. And high levels of betalains, taurine, calcium, magnesium, and antioxidants [36].

1.3.3. Importance

Prickly pear cactus fruits are very sweet and known in the countries over the entire world that harvest cactus. In the origin countries such as Mexico and Spain the young cladodes (2 years old / 15-20cm long) of some cactus species mainly *opuntia ficus*

indica are edible as fresh or cooked vegetable [35]. A research done in the target areas where this study relied on reveals that the major functions of cactus as famine food, short time occupation livestock feed (Cladodes and fruit peel), cash income, environmental protection (soil and water conservation), fence, fire wood, cochineal production and bee forage are more pronounced [40].

In herbal therapy it is used to lower fever and relieves chest pains, as a healing pad in cases of rheumatic and asthmatic symptoms of the chest, liver trouble, earaches, against diabetes, skin abrasions and tumors (skin growths that are not necessarily malignant), for cases of sun/wind burn, minor rash/burn, hemorrhoids, snake/insect bites, and minor abrasions, to treat diabetes, high cholesterol and obesity. Flavonoids present in the plant and its fruit can have neuroprotective effects, as well as antioxidant and free radical scavenging properties. It has also diuretic effect, treat gastric ulcers [36, 41].

1.4. Cochineal Insect

1.4.1. Overview

This insect belongs to Domain-Eukaryota, Kingdom - Animalia, Phylum - Arthropoda, Class - Insecta, Order - Hemiptera, Superfamily- Coccoidea, Family- Dactylopiidae, Genus - *Dactylopius*, Species - *D. coccus*. In addition, Cochineal (*Dactylopius coccus*) also called true cochineal is a scale-like insect that lives on cactus pads. The females are flat, oval-shaped and less than a quarter of an inch long. The males are much less than females and they have wings and they are mobile. The females, they attach to the cactus through their mouth and suck the juices. They also secrete white cotton-like balls or coatings on the cactus [42]. Out of the 200 species of *Opuntia* cacti from which cochineal can be cultivated, the most common is *Opuntia ficus-indica* [43].

1.4.2. Importance and Side Effect

Importance

Since long time mainly it is used as source of colouring agent. Carmine dye known by its commercial name as cochineal extract, "carmine", "natural red 4", or "natural colouring" is a natural, red colouring agent derived from the cochineal insect. Due to global restrictions on artificial colorants in food and other consumer items the carmine dye is used in foods, drugs, and cosmetics, beverages and for medical applications. Also it is not carcinogenic but it is more light and heat-stable with time than many synthetic dyes. Cochineal dye is still better as a dye for wool than most synthetics produced at much cheaper. Peru is the major supplier then Chile, then Canaries and at fourth Bolivia. Peru enjoys a considerable advantage in the world market, supplying 80% of the world's cochineal, about 40% as a dye and 60% in insect form. Cochineal is still produced and exported from Peru (200 tons/year). In 2005 when synthetic food dyes were selling for \$10-20 per kilogram, cochineal was selling for \$ 50-80 per kilogram [42].

Side effect

These insects are in fact parasites, and in very large numbers can damage the cactus. They are responsible for mortality of jointed cactus plants. The cochineal insect also limits spread and propagation of the plant by decreasing production of cladodes [42, 43, 44].

Therefore, the main objective of this work was to heal the infected cactus to the farmers in need of it with focus to investigate pesticide properties of the essential oil of *Eucalyptus camaldulensis* against cochineal insect.

2. Methodology

2.1. Materials and Methods

2.1.1. Materials

(i) Chemicals and Solvent

Glacial acetic acid, acetic anhydride, ammonium solution, conc. hydrochloric acid, sodium hydroxide, potassium hydroxide, sodium mercuric chloride, potassium iodide, calcium hydroxide ($\text{Ca}(\text{OH})_2$), iodine, potassium iodide, ferric chloride (FeCl_3), picric acid, concentrated sulphuric acid, dilute ammonia solution, n-hexane, diethyl ether, acetone, benzene, ethyl acetate, and ethanol.

(ii) Instruments and Equipment

UV (Ultraviolet-Visible Spectroscopy (for TLC view) thermometer, distiller, pestle and mortar, vacuum pump, separatory funnel, pipettes, burettes, Clevenger apparatus, condenser, three necked round bottom flask, heating mantle, rotatory evaporator, fridge, electrode beam balance were the major instruments used in this research work.

2.1.2. Methods

(i) Plant Material Collection

Eucalyptus camaldulensis fresh leaves was collected from field near to the research laboratory while, retrieved oils was used for the assay regarding *Eucalyptus globulus* [27].

(ii) Extraction of Essential Oil

Extraction was done after samples were washed with tap water and shade dried at room temperature for 8 -10days using hydro distillation in Clevenger type apparatus. The obtained essential oil was purified and stored under refrigerator for further use [27, 45, 46].

The percent yields of essential oil extracted was calculated as;

Percent yields = weight of the oil/weight of the sample used [46, 47]

(iii) Phytochemical Screening

Both *Eucalyptus camaldulensis* leaf and oil was tested for the presence of different secondary metabolites such as Phenol, alkaloids, quinones, flavonoids, tannins, steroids, saponins and terpenoids. The experiments carried out following standard phytochemical tests [46, 48].

(iv) Insecticidal Activity

Equal sizes of both adult and young female as well as male were collected carefully in glass and plastic bottles from the most infected area near to the research laboratory. Insecticidal activity test methods presented elsewhere was used with some modifications. In both male and female, insects of different age (young (30) and adult (10)) were put into the 2500 ml glass jars. Essential oil was then applied on a filter-paper strip measuring 3 x 3 cm attached to the lower side of the jar's lid. Doses were calculated based on nominal concentrations and assumed 100% volatilization of the oils in the exposure vessels. The first insecticidal activity experiments were conducted at constant temperature ($27 \pm 1^\circ\text{C}$), photoperiod (14L: 10D) and relative humidity ($60\% \pm 5$) for 24 h. While for the sake of obtaining the exact death and time of paralysis the second experiment was done out of the incubator. In either case insects of both ages were exposed to essential oil vapors (100, 80, 60, 40, 20, 10, 5, 3, 1 $\mu\text{l/l}$ air). A dose-mortality line was developed depending on the exposure time(s), and the lethal concentration of essential oil needed to kill 50% of the pest population (LC50) was also determined. Three replicates were set up for each dose and exposure time. In a small designed field containing six cladodes spraying was also done for three consecutive days at 100, 200, 300, 400, 500, 600 $\mu\text{l/air}$ of oil concentrations. All replicates were run simultaneously during the experiments. A complete set of controls was also maintained and replicated three times for each treatment [25, 26, 27, 49].

3. Result and Discussion

3.1. Description and Yield of the Plant

The scientific name, local name (in Tigrigna and Amharic), together with the essential oil yield of the plant is displayed in the table below.

Table 1. Scientific name, local name, description and essential oil yield of *Eucalyptus camaldulensis*.

| Scientific name | Local name (in Tigrigna and Amharic) | Characteristic appearance | Essential oil yield (%w/w) |
|---------------------------------|---|--|----------------------------|
| <i>Eucalyptus camaldulensis</i> | Tig: Keyh - Qelamitos, Amh: Key-Baharzaf | Stem-branched and dark brown; flower, cream-coloured white brown fibers; mature leaves, narrow, sickle-shaped and dark shining green; height, 20 - 40m high. ¹⁷ | 1.41(dry weight) |

3.2. Phytochemical Screening Findings

In studying medicinal value of a plant checking the presence of different secondary metabolites is very crucial and first step. The natural products listed for this study are all intentionally investigated because they have confirmed medicinal efficacy with known mechanism of action against various maladies [50, 51, 52, 53]. The presence of tannins, alkaloids and terpenoids in this plant oils was promising to proceed further for instance.

Table 2. Secondary metabolites present in *Eucalyptus camaldulensis* leaf and oil.

| S/No | Phytochemicals | <i>Eucalyptus camaldulensis</i> (oil) | <i>Eucalyptus camaldulensis</i> (leaf) |
|------|---------------------|---------------------------------------|--|
| 1 | Phenol | - | + |
| 2 | Alkaloids | ++ | - |
| 3 | Free anthraquinones | - | + |
| 4 | Flavonoids | - | - |
| 5 | Tannins | ++ | ++ |
| 6 | Steroids | ++ | ++ |
| 7 | Terpenoids | ++ | ++ |
| 8 | Saponins | ND | + |
| 9 | Cardiac glycosides | + | + |
| 10 | Volatile oil | ++ | ++ |

Key: (++)=strong presence, (+)=presence, (-)= absence, (ND)=not determined

3.3. Insecticide Activity on Cochineal Insect

3.3.1. *Eucalyptus Camaldulensis*

Compared to *Eucalyptus globulus* and those commercial pesticides studied before under the same situations higher insecticidal activity has recorded here. This specific eucalyptus species has yellowish color and strong aroma. The main mechanism of action by which the insects are expected to die is through this bad and toxic smell mainly from killing them up to decreasing their appetite towards sucking the plant until they die from starvation. Female insects eat too much than the male and they are also much bigger. According to the findings in the below two consecutive tables the males tend to be more susceptible to the oil compared to corresponding females of the same age. Based on their age the young tend to die before the adult at almost all concentrations (Table 3 and Table 4). In both cases the reason is direct. In the former one in addition to behavior of the females to stick to the cladode by their mouth (where the head part is covered) the size also matters. In the later as they are new to the environment and to survival tasks that they may easily affected by the oil.

Table 3. Paralysis and death time of *Eucalyptus camaldulensis* essential oils over young cochineal insect male and female.

| S/No | Treatment groups | Concentration | Cochineal Male | | Cochineal Female | |
|------|--------------------------|---------------|------------------|-------|------------------|-------|
| | | | Age(young) | | Paralysis | |
| | | | Time(in minuets) | | Death | |
| 1 | Eucalyptus camaldulensis | 100 | 20±8 | 38±10 | 49±16 | 80±15 |
| | | 80 | 27±6 | 37±7 | 54±11 | 90±43 |

| | | | | |
|----|--------|--------|--------|--------|
| 60 | 35±11 | 46±15 | 74±17 | 105±17 |
| 40 | 45±9 | 55±4 | 85±9 | 118±20 |
| 20 | 54±11 | 63±15 | 85±18 | 140±9 |
| 10 | 70±8 | 78±5 | 100±27 | 141±25 |
| 5 | 79±10 | 88±12 | 123±27 | 150±46 |
| 3 | 98±6 | 104±33 | 155±35 | 188±36 |
| 1 | 112±16 | 134±13 | 185±64 | 202±25 |

- Results on this biological study were reported as mean ± Standard deviation by formula. n= 30 in each group.

Table 4. Paralysis and death time of *Eucalyptus camaldulensis* essential oils over adult cochineal insect male and female.

| S/No | Treatment groups | Concentration | Cochineal Male | | Cochineal Female | |
|------|--------------------------|---------------|------------------|--------|------------------|--------|
| | | | Age(adult) | | | |
| | | | Time(in minuets) | | | |
| | | | Paralysis | Death | Paralysis | Death |
| 1 | Eucalyptus camaldulensis | 100 | 34±9 | 72±13 | 69±4 | 86±14 |
| | | 80 | 47±11 | 81±7 | 98±13 | 106±23 |
| | | 60 | 33±12 | 72±15 | 110±10 | 109±19 |
| | | 40 | 48±8 | 85±12 | 122±33 | 137±14 |
| | | 20 | 54±8 | 96±15 | 125±28 | 137±9 |
| | | 10 | 66±11 | 122±11 | 140±18 | 161±22 |
| | | 5 | 88±14 | 102±13 | 133±12 | 160±26 |
| | | 3 | 124±17 | 148±9 | 159±11 | 188±16 |
| | | 1 | 128±12 | 153±20 | 210±37 | 220±45 |

- Results on this biological study were reported as mean ± Standard deviation by formula. n= 10 in each group.

Table 5. Paralysis and death time of *Eucalyptus globulus* and *Eucalyptus camaldulensis* essential oils over young cochineal insect male and female.

| S/No | Treatment groups | Concentration | Cochineal Male | | Cochineal Female | |
|------|--|---------------|------------------|--------|------------------|--------|
| | | | Age(young) | | | |
| | | | Time(in minuets) | | | |
| | | | Paralysis | Death | Paralysis | Death |
| 1 | Eucalyptus camaldulensis and Eucalyptus globulus | 100 | 31±14 | 52±21 | 67±19 | 77±29 |
| | | 80 | 32±11 | 66±20 | 70±20 | 85±24 |
| | | 60 | 32±17 | 59±20 | 90±24 | 104±33 |
| | | 40 | 48±18 | 67±17 | 100±15 | 127±35 |
| | | 20 | 76±22 | 82±25 | 140±18 | 158±26 |
| | | 10 | 75±24 | 99±19 | 156±35 | 174±48 |
| | | 5 | 72±18 | 95±20 | 160±36 | 178±28 |
| | | 3 | 98±11 | 132±12 | 205±22 | 274±51 |
| | | 1 | 122±24 | 182±5 | 200±32 | 286±65 |

- Results on this biological study were reported as mean ± Standard deviation by formula. n= 30 in each group.

3.3.2. *Eucalyptus Globulus* and *Eucalyptus Camaldulensis* (50:50)

For the sake of knowing the synergetic effect and use it as an advantage; essential oil mixture of both eucalyptus varieties was also used as one treatment agent. In almost all of the concentrations the mixture found out to be less potent than individual oil extracts of the species even though, the recordings are significant and higher compared to the commercial pesticide agents. This was true for female and males in their young and adult age state.

Table 6. Paralysis and death time of *Eucalyptus globulus* and *Eucalyptus camaldulensis* essential oils over adult cochineal insect male and female.

| S/No | Treatment groups | Concentration | Cochineal Male | | Cochineal Female | |
|------|--|---------------|------------------|-------|------------------|--------|
| | | | Age(adult) | | | |
| | | | Time(in minuets) | | | |
| | | | Paralysis | Death | Paralysis | Death |
| 1 | Eucalyptus 50tus camaldulensis and Eucalyptus globulus (50:50) | 100 | 48±9 | 54±16 | 71±8 | 85±21 |
| | | 80 | 43±14 | 62±18 | 89±8 | 95±13 |
| | | 60 | 62±11 | 79±19 | 87±14 | 94±10 |
| | | 40 | 74±11 | 89±8 | 98±17 | 133±12 |
| | | 20 | 92±9 | 104±5 | 120±10 | 140±14 |

| | | | | |
|----|--------|--------|--------|--------|
| 10 | 87±24 | 100±19 | 144±21 | 174±19 |
| 5 | 78±20 | 99±14 | 146±16 | 168±18 |
| 3 | 121±10 | 122±12 | 146±12 | 154±21 |
| 1 | 139±22 | 158±11 | 195±36 | 246±55 |

- Results on this biological study were reported as mean ± Standard deviation by formula. n= 10 in each group.

3.4. Lethal concentration of Treatment Groups

Lethal concentration at 50% inhibition (LC50) values were calculated from graph in Microsoft excel soft were. All the concentrations were real with acceptable regression value for scientific experiments ($R^2 > 0.7$) (Table 7).

Table 7. Lethal concentration of treatment groups needed to kill 50% of the insects.

| S/N | Agents | Cochineal insects | | LC50 | R ² | Equation |
|-----|--|-------------------|-------|-------|----------------|-------------------------|
| | | Sex | Age | | | |
| 1 | Eucalyptus camaldulensis | Male | Young | 43.56 | 0.714 | $y = -0.75x + 98.105$ |
| | | | Adult | 78.34 | 0.651 | $y = -0.6789x + 127.5$ |
| | | Female | Young | 68.52 | 0.8207 | $y = -1.024x + 171.17$ |
| | | | Adult | 69.21 | 0.8035 | $y = -1.033x + 181.5$ |
| 2 | E. camaldulensis & E. globulus (50:50) | Male | Young | 37.4 | 0.561 | $y = -0.8476x + 122.71$ |
| | | | Adult | 59 | 0.746 | $y = -0.736x + 122.43$ |
| | | Female | Young | 46.51 | 0.734 | $y = -1.7673x + 225.2$ |
| | | | Adult | 53.27 | 0.7086 | $y = -1.158x + 184.27$ |

3.5. Percent Inhibition of Cochineal Insect on Spray

On spay test concentration were increased higher from the maximum concentrations used in the laboratory. This is due to high volatility of the oils. Eucalyptus camaldulensis alone was effective compared to its synergetic effect together with globulus. Specifically, during the spray, the number of the insects of both sexes was estimated to be more than 250 and quick paralysis was seen more in the cladode sprayed with Eucalyptus camaldulensis.

3.5.1. Percent of Inhibition

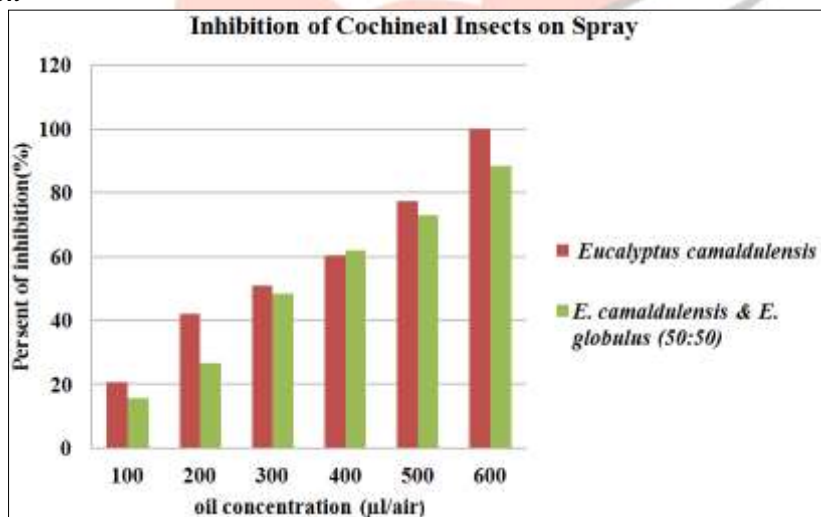


Fig. 3.1. Inhibition of cochineal insect on spray.

3.5.2. Lethal Concentration (LD50)

Table 8. Lethal concentration (LD50), Equation and R² values of treatment groups on spray.

| S/N | Treatment groups | LD50 | R ² | Equation |
|-----|--|---------|----------------|------------------------|
| 2 | Eucalyptus camaldulensis | 291.524 | 0.976 | $y = 0.1467x + 7.2333$ |
| 3 | E. camaldulensis & E. globulus (50:50) | 334.234 | 0.9912 | $y = 0.148x + 0.5333$ |

4. Conclusion

In natural product research it is common to study many plants in search for better efficacy. Here at all assays employed Eucalyptus camaldulensis proved to be more potent than corresponding globulus and the commercial pesticides. The secondary metabolites existing in the plant are all with inhibiting capacity. Unlike in most insecticidal assays which used 1000L jars the

volume of the jars in this study has increased to 2500L. This shows the strong aroma of the plant to repel and inhibit the insects. This was confirmed both in the laboratory and field spray tests. The findings are in agreement with outstanding data recorded in another studies. Moreover, the oils are environment friendly, not harmful when handled and volatile. In addition to its use as a weapon against the cochineal locally it is also common remedy for cough and breathing related illness. Having the oil in their hand the peoples have also such health benefits. This is a serious issue. Peoples have begun to complain the regional government. Attempts are ongoing to deal with the problem but they are not enough. Here we have reached at hoping results that if there is a push from the university or the regional state we could heal the cactus in very short time. Oils can also appear in the market for this and other public purposes.

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References

- [1] Griffith, M. P. (2004). The origins of an important cactus crop, *Opuntia ficus indica* (Cactaceae): New molecular evidence. *American Journal of Botany*, 91, 1915–1921
- [2] Neumann, L., Opening speech. In: Proceedings of the International Workshop on “*Opuntia* in Ethiopia: State of Knowledge in *Opuntia* Research” February 23-27, 1997, Mekelle University, Ethiopia and Wiesbaden Polytechnic, Germany.
- [3] Habtu, L. (2005). Cactus in southern Tigray: Current status, potential uses, utilization and threat. M.Sc. Thesis, Addis Ababa University. [unpublished].
- [4] Ben Salem, H., Nefzaoui, A., Abdouli H. and Orskov, E. R. Effect of increasing level of spineless cactus (*Opuntia ficus-indica* var. *inermis*) on intake and digestion by sheep given straw based diets. *Journal of Animal Science*, 62, 293-299, 1996.
- [5] Barbera, G. “History, economic and agro-ecological importance: Agro-ecology, cultivation and uses of cactus pear,” FAO Plant Production and Protection Paper, 132, 1-11, 1995.
- [6] Firew, T. “Evaluation of alternative feed resources for ruminants under arid zones of the tropics and sub-tropics: The case of cactus pear (*Opuntia ficus indica*) in Ethiopia,” Ph. D Thesis, Humboldt University of Berlin, Germany, 2007.
- [7] Nefzaoui, A., Inglese P. and Belay, T. “Improved utilization of cactus pear for food, feed, soil and water conservation and other products in Africa (Eds). Proceedings of International Workshop, 19- 21 October 2009. Mekelle, Ethiopia, 224, 2010.
- [8] Mohamed, G. A. R., Ibrahim M. S. and Eucalyptone, G. (2007) A new phloroglucinol derivative and other constituents from *Eucalyptus globulus* Labill.. *ARKIVOC*, xv, 281-291
- [9] Ghalem B. R. and Mohamed, B. (2014). Antibacterial activity of essential oil of North West Algerian *Eucalyptus camaldulensis* against *Escherichia coli* and *Staphylococcus aureus*. *Journal of Coastal Life Medicine*, 2(10), 799-804
- [10] Elaissi, A., Rouis, Z., Salem, N.A., Mabrouk, S., Salem, Y., Haj Salah, K. B., Aouni, M., Farhat, F., Chemli, R., Harzallah-Skhiri, F. and Khouja, M. L. (2012). Chemical composition of 8 eucalyptus species' essential oils and the evaluation of their antibacterial, antifungal and antiviral activities. *BMC Complementary Alternative Medicine*, 12(81), 1-15
- [11] Kumar, H.D. and S. Laxmidhar. (2011). A review on phytochemical and pharmacological of *Eucalyptus globulus*: A multipurpose tree. *International Journal of Research in Ayurveda Pharmacy*, 2(5), 1527-1530
- [12] Godghate, A.G. and Sawant, R.S. (2014) Secondary metabolites determinations qualitatively from bark of *butea monosperma* and *Eucalyptus globulus*. *International Journal of Science and Environmental Technology*, 3(2), 497 – 501
- [13] Chhetri, H. P., Yogol, N.S., Sherchan, J., Anupa, K.C., Mansoor, S. and Thapa, P. (2008). Phytochemical and antimicrobial evaluations of some medicinal plants of Nepal. *Kathmandu University Journal of Science. Eng.Tech.* 1 (V), 49-54
- [14] Yuvneet, R., Navneet, K., Deepa, A., Rajandeep K. and Hatish, P. (2013). Phytochemical analysis and antimicrobial activity of methanolic extract of *Eucalyptus globules*. *Journal of Microbiology and Biotechnology Research*, 3 (2), 77-82
- [15] Damjanović-Vratnica, B., Đakov, T., Šuković, D. and Damjanović, J. (2011). Antimicrobial Effect of Essential Oil Isolated from *Eucalyptus globulus* Labill. from Montenegro. *Czech Journal of Food Science*, 29(3), 277–284
- [16] Sani, I., Abdulhamid A. and Bello, F. (2014). *Eucalyptus camaldulensis*: Phytochemical composition of ethanolic and aqueous extracts of the leaves, stem-bark, root, fruits and seeds. *Journal of Science Innovation Research*, 3(5), 523-526
- [17] Siramon, P., Ohtani Y., and Ichiura, H. (2013). Chemical Composition and Antifungal Property of *Eucalyptus camaldulensis* Leaf Oils from Thailand. *Records of Natural Products*, 7(1), 49-53
- [18] Mubarak, E. E., Ali, L.Z., Ahmed, I.F.A., Ahmed A.B.A. and Taha, R.M.(2015). Essential Oil Compositions and Cytotoxicity from Various Organs of *Eucalyptus camaldulensis*. *International Journal Of Agriculture & Biology*, 17(2), 320-326
- [19] Elaagib, E., Taha R.M. and Mohajer, S. (2012). Chemical Composition and Antimicrobial Activity of *Eucalyptus camaldulensis* Dehnh Essential Oil from Malaysia. *Malaysian Journal of Pharmacy*. 1(10), 111-115

- [20] Dixit, A., Rohilla, A. and Singh, V. (2012). Eucalyptus globulus: A New Perspective in Therapeutics. *International Journal of Pharmacy and Chemical Sciences*, 1 (4), 1678-1683
- [21] Raho, G. B. and Benali, M. (2012). Antibacterial activity of the essential oils from the leaves of *Eucalyptus globulus* against *Escherichia coli* and *Staphylococcus aureus*. *Asian Pacific Journal of Tropical Biomedicine*, 2(9), 739-742
- [22] Boukhatem, M.N., Amine, F.M., Kameli, A., Saidi, F., Walid, K. and Mohamed, S.B. (2014). Quality assessment of the essential oil from *Eucalyptus globulus* Labill. of Blida (Algeria) origin. *International Journal of Biomedical and Biological Engineering*, 17(3), 303-315
- [23] Gende, L., Maggi, M., Van Baren, C., Leo Lira, A., Bandoni, A., Fritz R., and Eguaras, M. (2010). Antimicrobial and miticide activities of *Eucalyptus globulus* essential oils obtained from different Argentine regions. *Spanish Journal of Agricultural Research*, 8(3), 642-650
- [24] El-Moein, N. M., Mahmoud E. A. and Shalaby, E. A. (2012). Antioxidant Mechanism of Active Ingredients Separated from *Eucalyptus globulus*. *Organic Chemistry Current Researches*, 1(2), 1-7
- [25] Mousa, K. M., Khodeir, I. A., El-Dakhkhni, T.N. and Youssef, A. E. (2013). Effect of Garlic and Eucalyptus oils in comparison to Organophosphate insecticides against some Piercing-Sucking Faba bean insect Pests and natural enemies' populations. *Egypt Academia Journal of Biological Sciences*, 5, 21 -27
- [26] Yang, Y.C. Lee, H.S., Clark, J. M. and Ahn, Y. J. (2004). Insecticidal Activity of Plant Essential Oils Against *Pediculus humanus capitis* (Anoplura: Pediculidae). *Journal of Medical Entomology*, 41, 700-704
- [27] Maciel, M.V., Morais, S.M., Bevilaqua, C.M., Silva, R. A., Barros, R. S., Sousa, R. N., Sousa L.C., Brito E.S. and Souza, M. A. (2010). Chemical composition of *Eucalyptus* spp. essential oils and their insecticidal effects on *Lutzomyia longipalpis*. *Veterinary Parasitology*, 167, 1-7
- [28] Musa, D. A., Nwodo, F.O.C. and Ojogbane, E.(2011). Phytochemical, antibacterial and toxicity studies of the aqueous extract of *Euclayptus camaldulensis* Dehnh. *Asian Journal of Plant Science Research*, 1(3), 1-10
- [29] Abubakar, E.M. (2010). Antibacterial potential of crude leaf extracts of *Eucalyptus camaldulensis* against some pathogenic bacteria. *African Journal of Plant Science*, 4(6), 202-209
- [30] Meshkani, N., Naghsh, N. and Ranjbar, M. (2014) Study of cytotoxic effects of Ethanolic extract of *Eucalyptus camaldulensis* leaf on the cells k562 of human chronic Myelogenous leukemia (CML) under in Vitro conditions. *Bulletin of Environmental Pharmacology and Life Sciences*, 3(III), 186-190
- [31] Lima, L. M., Babakhani, B., Boldaji, A. S.H., Asadi M. and Boldaji, R. M. (2013). Essential oils composition and antibacterial activities of *Eucalyptus camaldulensis* Dehn. *International Journal of Medicinal and Aromatic Plants*, 3(2), 214-219
- [32] Pandey, B. and Singh, S. (2014). Evaluation of Antimicrobial Potential of *Eucalyptus camaldulensis* L. *Journal of Pharmaceutical, Chemical and Biological Sciences*, 2(3), 166-171
- [33] Akin, M., Aktumsek, A. and Nostro, A. (2010). Antibacterial activity and composition of the essential oils of *Eucalyptus camaldulensis* Dehn. and *Myrtus communis* L. growing in Northern Cyprus. *African Journal of Biotechnology* 9 (4), 531-535
- [34] Igueras, G. A. L. and Ortillo, L. P. (2001). Uses of *Opuntia* Species and the Potential Impact of *Cactoblastis Cactorum* (*Lepidoptera: Pyralidae*) In Mexico. *Florida Entomology*, 84(4), 493-498
- [35] Rodriguez-Felix, A., Cantwell, M. and Robles-Contreras, F. (1992). Postharvest physiology of prickly pear cactus stems. *Scientia Horticulture*, 50, 1-9
- [36] Piga, A.(2004). Cactus Pear: A Fruit of Nutraceutical and Functional Importance, *J. PACD*, 9-22
- [37] El-Samahy, S. K., Abd El-Hady, E. A., Habiba, R. A. and Moussa-Ayoub, T. E. (2007). Some Functional, Chemical, and Sensory Characteristics of Cactus Pear Rice-Based Extrudates.136-147
- [38] Nassar, A.G. (2008). Chemical Composition and Functional Properties of Prickly Pear (*Opuntia ficus indica*) Seeds Flour and Protein Concentrate. *World Journal of Dairy & Food Science*, 3 (1), 11-16
- [39] A. Rodriguez-Felix and M. Cantwell, "Developmental changes in composition and quality of prickly pear cactus cladodes (nopalitos). Palnt foods for human nutrition," 38, 83-93, 1988.
- [40] Gebretsadik, G., Anmut, G., Tegegne, F. (2013). Assessment of the potential of cactus pear (*Opuntia ficus indica*) as livestock feed in Northern Ethiopia. *Livestock Research for Rural Development*, Chemical Abstract, 25 (2) 2), [Online] Available: <http://www.lrrd.org/lrrd25/2/moen25026.htm>
- [41] Butera, D., , Tesoriere, L., Di Gaudio, F., Bongiorno, A., Allegra, M., Pintaudi , A. M., Kohen, R. and Livrea, M. A.(2002). "Antioxidant activities of sicilian prickly pear (*Opuntia ficus indica*) fruit extracts and reducing properties of its betalains: betanin and indicaxanthin. *Journal of Agriculture and Food Chemistry*, 50(23), 6895-6901
- [42] Weniger, D. (1991). *Cacti of Texas*. The University of Texas Press
- [43] BadII, M.H. and Flores, A. E. (2001). Prickly Pear Cacti Pests and Their Control In Mexico. *Florida Entomology*, 84(4), 503-505
- [44] <http://www.arc.agric.za/arcpri/Fact%20Sheets%20Library/Cochineal%20insects%20Dactylopius%20spp.pdf>. (Google home page, accessed December 2015).
- [45] Tiwari, P., Kumar, B., Kaur, M., Kaur, G. and Kaur, H. (2011).Phytochemical screening and Extraction: A Review. *International pharmaceutica sciencia*, 1,103-104.
- [46] Saim, N., Osman, R., Hirni Md Yasin, A. and Hamid, R. (2008). Subcritical water extraction of essential oil from coriander (*Coriandrum sativum* L.) seeds. *The Malaysian Journal of Analytical Science*, 12, 1-2
- [47] Fabiane, K., Ferronato, R., Santos, A. and Onofre, B. (2007). Physicochemical characteristics of the essential oil of *Baccharis dracunculifolia* and *Baccharis uncinella* D.C. (Asteraceae). *Journal of Pharmacognocny*, 18, 197

- [48] Celikel, N. and Kavas, G. (2008). Antimicrobial Properties of Some Essential Oils against Some Pathogenic Microorganisms. *Czech Journal of Food Science*, 6, 174–181
- [49] Cipolca, L. (2005). Chemistry of Natural Product Compounds. *Organic and Bio molecular Chemistry*, 3, 2-3
- [50] Jeyachandran, R., Mahesh, A., Cindrella, L., Sudhakar, S. and Pazhanichamy, K. (2009). Antibacterial Activity of Plumbagin and Root Extracts of *Plumbago Zeylanica* L. *Seri. Botanica*. 51, 17–22
- [51] Kashiwada, Y., Nonaka, G., Nishioka, I., Chang, J. J. and Lee, K.H. (1992). Antimour agents, 129. Tannins and related compounds as selective cytotoxic agents, *Journal of Natural Products*, 55, 1033 -1043
- [52] Paiva, R. S., Figueiredo, R. M., Aragão, V. T. and Kaplan, C. A. M. (2003). Antimicrobial Activity in Vitro of Plumbagin Isolated from *Plumbago* Species. *Mem Inst Oswaldo Cruz*, 98, 959-961
- [53] Jagessar, C. R. and Cox, M. (2010). Phytochemical Screening of the CHCl_3 and $\text{CH}_3\text{CH}_2\text{OH}$ Extract of Stems, Twigs, Roots and Barks of *Conocarpus Erectus*. L. *International Journal of Academic Research*, 2, 36-45

