Global Journal of Science Frontier Research: B
Chemistry
Volume 15 Issue 5 Version 1.0 Year 2015
Type : Double Blind Peer Reviewed International Research Journal
Publisher: Global Journals Inc. (USA)
Online ISSN: 2249-4626 & Print ISSN: 0975-5896

Determination of Phytochemical Constituents and Insecticidal Activity of Eucalyptus Globules Against Cactus Pests

By Yohannes Weldemariam & Shishay Welderufael
Adigrat University, Ethiopia

Abstract- Cactus pear is widely distributed in Tigray and South Amhara Regional state and is the integral part of the economy of the people. It is commonly used as a famine food, feed for livestock, bee forage, a source of cash income and short time occupation, soil and water conservation, live fences etc. However, cactus is been gradually destroyed. The peoples relied on cactus are suffering from the destruction. The effect of the insect *cochineal* has been an alarm around the region. The intention of this research was to find a solution to insects and pests on cactus. Insecticidal activity was done on *Eucalyptu* soil extracts and on three commercial pesticides. At all concentrations the oils terminate the cochineal insects of all ages. Especially, the male cochineals (adult and young) and young female were more sensitive to the oils respectively. Findings from spray test are promising with 80-90% inhibition in average.

Keywords: opnitiaficusindica, eucalyptus globulus, hydro distillation, essential oil, phytochemical screening, physicochemical constants, insecticidal activity.

GJSFR-B Classification : FOR Code: 259999p

Strictly as per the compliance and regulations of :

Determination of Phytochemical Constituents and Insecticidal Activity of Eucalyptus Globules Against Cactus Pests

Yohannes Weldemariam [α] & Shishay Welderufael [σ]

Abstract- Cactus pear is widely distributed in Tigray and South Amhara Regional state and is the integral part of the economy of the people. It is commonly used as a famine food, feed for livestock, bee forage, a source of cash income and short time occupation, soil and water conservation, live fences etc. However, cactus is been gradually destroyed. The peoples relied on cactus are suffering from the destruction. The effect of the insect *cochineal* has been an alarm around the region. The intention of this research was to find a solution to insects and pests on cactus. Insecticidal activity was done on *Eucalyptu s*oil extracts and on three commercial pesticides. At all concentrations the oils terminate the cochineal insects of all ages. Especially, the male cochineals (adult and young) and young female were more sensitive to the oils respectively. Findings from spray test are promising with 80-90% inhibition in average.

Keywords: opnitiaficusindica, eucalyptus globulus, hydro distillation, essential oil, phytochemical screening, physicochemical constants, insecticidal activity.

I. Introduction

Unlike those edible vegetable oils essential oil(ethereal oil) is a concentrated, hydrophobic liquid that contains hundreds of aromatic compounds, organic constituents, and other natural elements found in dried or fresh leaves, stems, flowers, bark, wood, roots or other elements of a plant(Buchbauer2010). Essential oils has been used for psychological and physical wellbeing since long timein China and Egypt. Yet, its medicinal potential was known only during the earlier part of the 20thCentury as discovered by French Chemist Rene-Maurice Gattefosse(Buchbauer2010,Taylor and Francis Group 2010,Cipolca2005, Celikel and Kavas 2008).

Essential oils can be used for treating burned skin or to heal wounds due to their anti-inflammatory properties (Derwich et al. 2010). They also bear role to play in the protection of crops against mould, mildew and wood root fungi and plants against various insects (Isman 2000). In addition, when applied in a vapor form, essential oil has potential to manage weeds, especially as its toxicity appears to be harmful to the insects. Since essential oils are a complex mixture of chemical components their synergetic effect is promising. Most of them work in the same way as:

- Repellents which drive the insects away from the plant by their smell or taste
- Anti- feed ants which cause insects feeding on the plants to reduce their food intake until they die from starvation
- Ovipositor deterrents which prevent insects from laying eggs
- Inhibitors which stop the development of different stages of the insect(Isman 2000, Hussain2009, Can Baser and Buchbauer 2010).

Globules oil *from Eucalyptus* genus can control aphids, Piercing-Sucking insect pests associated with Faba bean *Viciafaba by* reducing the population of leafhoppers and planthoppers (Mousa et al. 2013). Data from insecticidal activity of this plant indicates it is lethal to female Pediculushumanuscapitis De Geer insect (Yang et al. 2004).On another study it has lethal effect to Sternechus subsignatus, Rhyssomatussubtilis and Lutzomyialongipalpis, with significant mortality (contact toxicity (LD50 = 0.40 and 0.84 μL/cm^2 for S. pinguis and R. subtilis respectively) two important pest of soybean (Maciel et al.2010).

Cactus pear is widely distributed in Tigray Regional State and is the integral part of the economy of the people. Fruits are very sweet and have many essential components comparable to mostly used fruits and known in the countries over the entire world where cactus grows. Young cladodes are edible as fresh or cooked vegetable in the origin county Mexico and other countries. As reported in one study done on uses of cactus as livestock feed in Northern Ethiopia it revealed many uses such as famine food, feed for livestock, bee forage, a source of cash income and short time occupation, soil and water conservation, live fences etc (Gebretsadik2013). With regard to its medicinal application 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 and neuro protective effects. However, here in Tigray its production and quality as compared to its potential is very low. One of

Author α: Department of Chemistry, Adigrat University, Adigrat, Ethiopia, e-mail: jonygsfusw@gmail.com
Author σ: Department of Plant Science, Adigrat University, Adigrat, Ethiopia. e-mail: shushay24@gmail.com

the main reasons is damage of insect and disease. This fruit crop can be affected by disease such as Phyllosticta pad spot, Anthracnose, *Echinocereus, Pythium*, Sammons'virus, Bacterial necrosis and insects like *Scyphophorusacupunctatus*, Cochineal scale, Cactus longhorn beetle, Coccid scale etc. despite of this fact, there are no research reports on the control methods of these insects and diseases which hindering its production and quality (Buchbauer2010, Derwich et al. 2010, Isman 2000, Mousa et al. 2013).The aim of this research project was to extract essential oil from one eucalyptusspecies as an pesticide specifically with focus to Cochineal insect. This is the first project to try to eliminate cochineal insects in natural chemical pesticide.

II. Methodology

a) Materials and Methods

i. *Materials*

a. *Chemicals and Solvent*

Glacial acetic acid, acetic anhydride, ammonium solution, conc. hydrochloric acid, sodium hydroxide, potassium hydroxide, sodium mercuric chloride , potassium iodide , calcium hydroxide ($Ca(OH)_2$), 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.

b. *Instruments and Equipment*

Thermometer, distiller, pestle and mortal, vacuum pump, separatory funnel. pipettes, burettes, Clevenger apparatus, round bottom flask, fridge, electrode beam balance were the major instruments used in this work.

ii. *Methods*

a. *Extraction of Essential Oil*

Fresh collected samples were washed with tap water, to eliminate soil and other surface contaminants and shade dried at room temperature for 8 -10days.The dried sample was crashed to increase surface area using pestle and mortal(Saim et al. 2008).The essential oil was then extracted from samples of *Eucalyptus* using hydro distillation in Clevenger type apparatus through the following procedure; first 300g of the sample was measured using beam balance and put in to 5000 ml three-necked round bottom flask using spatula. Then ¾ of flask was filled with distilled water. The round bottom flask was then placed on heating mantle and using metal clamps it was connected with the Clevenger apparatus and condenser. Then the flask was heated for 3-4hours. While it was heating the oil and water evaporated as stem. The oil appears at the top of the water. The essential oil was obtained by removing the water until it riches the layer of the oil. The obtained essential oil was purified and stored under refrigerator for further use (Maciel et al. 2010, Saim et al. 2008, Fabiane et al. 2007).

The percent yields of essential oil extracted was calculated as;

Percent yields = weight of the oil/weight of the sample used(Fabiane et al. 2007,Tiwari et al 2011)

b. *Insecticidal activity*

In order to test the toxicity of essential oil on the insects of different age (young and adult). Ten adults 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 was calculated and assumed 100% volatilization of the oils in the exposure vessels. The same procedure was repeated with only difference in the number of treatment groups that 30 young insects were used in both male and female. The first insecticidal activity experiments was conducted at constant temperature (27 ± 1°C), photoperiod (14L: 10D) and relative humidity (60% ± 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 μl/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 with all the treatment groups at (100, 200, 300, 400, 500, 600 μl/l air). All replicates were run simultaneously during the experiments. A complete set of controls was also be maintained and replicated three times for each treatment (Mousa et al. 2013, Yang 2004 et al, Macielet al2010).

III. Result and Discussion

a) Essential oil yield

From fresh leaf samples of *Eucalyptus globulus* 1.56(%w/w) of oil was obtained.

b) Phytochemical screening findings

For a plant to have phytochemical constituents means that the plant is promising to be used in as herbal medicine. Some of those determined here have clear mechanism of action with respect to the strains. The phenols are expected to be toxic though they absent from the essential oil. Alkaloids are known to act in central nervous system of the insects while tannins complex with proteins through so-called nonspecific forces such as hydrogen bonding and hydrophobic effects, as well as by covalent bond formation. They inactivate microbial adhesions, enzymes, and cell envelope transport etc (Jeyachandran et al. 2009, Kashiwada1992, Paiva et al. 2003, Jagessarand Cox, 2010, Schulzet al. 2004)Condensed tannins act by

binding to cell walls of for instance ruminal bacteria resulting in declining growth weakened activity. In the case of insects tannins specifically those condensed ones are expected to act the same.

Table 1 : Phytochemical constituents of *Eucalyptus globulus and Eucalyptus camaldulensis* leaf and oil.

S/No	Phytochemicals	*Eucalyptus globulus(oil)*	*Eucalyptus globulus(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

c) *Insecticide activity on cochineal insect*

In Cochineal insect taxonomy the female is about five times bigger than the male and it lacks wings. 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. Since the insects are host specific that it is impossible to grow them from egg level. We tried to collect them along with the cactus cladode. Insecticidal tests were also done while the insects are in the cladode and in isolated manner. The problem is the females are fixed to the cladode attached by their mouth. For the first time cochineal female and male (young and adult) were also tested with the treatment groups in an incubator for 24 hours. The next day none of the insects survive in the jars containing them. But we were also interested at determining the exact time when the insects will die and follow how they behave towards the oil from the beginning. Thus, we tested the insects in open but protected environment in the laboratory. The recorded data are as follows.

i. *Eucalyptus globulus*

The oils of this specific species of eucalyptus tend to paralyze and kill the insects. At all concentrations the recorded activity was dose dependent. At the highest concentration(100μL/air) young cochineal male paralyzed and dead at 29±9 and 49±12 respectively while, the females are bigger and stronger than the males that they get paralyzed and killed at some longer time(58±19 and 89±18). In the same sex the adult tend to resist more than the young. Because of this 30 young cochineal was included in each jar. Even at this number, the oils paralyze and kill with less time compared to the adult insects. Table 2 and table 3 display this finding.

Table 2 : Paralysis and death time of *Eucalyptus globulus* essential oils over young cochineal insect male and female

S/No	Treatment groups	Concentration(μL)	Cochineal Male		Cochineal Female	
			Age(young)			
			Time(in minuets)			
			Paralysis	Death	Paralysis	Death
1	*Eucalyptus globulus*	100	29±9	49±12	58±19	89±18
		80	33±11	50±14	70±24	99±17
		60	39±10	58±11	87±20	107±21
		40	51±20	75±17	97±25	126±38
		20	60±7	87±20	100±18	124±34
		10	77±11	99±18	107±23	142±30
		5	94±8	108±21	144±54	157±33
		3	121±25	148±37	164±43	189±41
		1	140±22	166±41	191±48	217±55

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

Table 3 : Paralysis and death time of *Eucalyptus globulus* 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	*Eucalyptusglobulus*	100	38±11	69±17	68±21	200±55
		80	45±7	88±12	100±30	185±33
		60	45±8	100±16	122±40	199±34
		40	62±14	99±14	200±39	253±64
		20	66±13	107±19	220±33	308±56
		10	82±11	134±20	267±68	552±80
		5	91±8	158±38	388±84	608±88
		3	111±21	197±36	441±55	789±91
		1	139±18	254±31	710±98	920±105

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

ii. *Commercial pesticides*

In case of an emergency there is a habit in many people all around the world to use these pesticides to manage mainly infections at house level. The aim of this specific assay was not to compare and contrast the activity of the essential oils with these pesticides considering them as reference standards. Rather it is to show the effect of the pesticides to the insects as well as to the plants taking them as single individual treatment agent. The time they took for paralysis and death of the insects is lower but comparable to the essential oils. So, they can also be recommended to be used. The major side effect seen was the outer layer of the cladode turned yellow sometime after of the spray. Since they are all chemicals they leave residue this can then affect the plant time from time. The effect to the animals after eating those sprayed cladodes any time after the spray is another major concern. As shown in the table below, males are eliminated at less time before females. The inhibition was dose dependent that with increasing concentration paralysis and death decreased. Yong cochineal insect of both sex tend to be moresusceptible than corresponding adult.

Table 4 : Paralysis and death time of Drusban (48%), Malathion(48%), Ventazion /Diainon(60%) over young cochineal insect male and female

S/No	Treatment groups	Concentration(μL)	Cochineal Male		Cochineal Female	
			Age(young)			
			Time(in minuets)			
			Paralysis	Death	Paralysis	Death
1	Drusban (48%)	100	36±16	55±11	71±14	96±13
		80	52±13	68±9	88±15	104±22
		60	42±16	77±9	101±10	125±24
		40	72±17	88±16	98±8	108±14
		20	100±10	111±12	128±21	150±20
		10	99±24	124±22	167±17	188±35
		5	145±33	154±25	164±20	185±35
		3	148±17	166±31	174±41	201±16
		1	172±33	204±38	225±19	263±44
2	Malathion(48%)	100	56±23	77±10	84±21	89±25
		80	68±14	94±14	88±15	102±28
		60	75±17	89±15	103±32	124±18
		40	86±24	97±32	138±14	158±46
		20	94±25	107±34	118±23	157±34
		10	125±16	142±26	188±28	209±31
		5	144±39	175±28	195±35	244±33
		3	164±47	180±31	211±43	239±26

		1	177±21	186±23	208±51	254±46
3	Ventazion /Diainon(60%)	100	68±22	75±16	96±28	130±42
		80	81±32	98±16	116±26	129±27
		60	92±27	105±15	110±20	145±34
		40	118±26	149±22	141±32	156±27
		20	133±42	150±24	179±19	188±26
		10	166±29	187±37	208±44	238±46
		5	195±25	206±38	258±17	277±56
		3	222±24	228±33	219±25	286±46
		1	232±19	248±44	304±49	337±55

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

Table 5 : Paralysis and death time of Drusban (48%), Malathion(48%), Ventazion /Diainon(60%) over adult cochineal insect male and female

S/No	Treatment groups	Concentration(µL)	Cochineal Male		Cochineal Female	
			Age(adult)			
			Time(in minuets)			
			Paralysis	Death	Paralysis	Death
1	Drusban(48%)	100	71±17	86±28	99±24	119±18
		80	84±27	108±19	97±22	127±32
		60	83±16	101±29	121±11	142±22
		40	79±24	91±36	140±18	160±17
		20	84±14	98±18	109±30	148±33
		10	102±18	116±36	108±26	138±42
		5	97±23	122±20	147±20	164±46
		3	104±17	128±32	130±31	161±22
		1	134±44	158±42	178±37	211±48
2	M alathion(48%)	100	84±23	96±21	94±24	108±23
		80	98±21	99±24	102±18	144±27
		60	111±22	128±22	148±39	155±15
		40	126±24	136±41	138±27	162±19
		20	137±23	149±19	172±44	187±34
		10	156±19	169±28	200±36	257±54
		5	148±34	170±11	204±35	266±38
		3	199±53	239±42	259±33	277±32
		1	222±32	256±21	247±46	271±31
3	Ventazion /Diainon(60%)	100	102±34	118±19	109±28	134±33
		80	122±22	139±29	128±19	159±29
		60	124±31	138±22	142±17	168±37
		40	128±16	133±32	153±22	168±27
		20	161±50	174±36	201±11	241±18
		10	164±29	194±19	189±53	266±37
		5	184±42	193±28	184±52	246±29
		3	237±58	249±48	276±33	298±26
		1	258±29	269±39	296±56	328±38

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

d) Lethal concentration of treatment groups

For the sake of complying with statistical validity of the data, lethal concentration at 50% inhibition (LC50) values were calculated from graph in Microsoft excel. The equations for these graphs are shown in (Table 12). When the equations are worked out for "X" value the results are correlated to LC50values. The R^2 values confirm the validity of the data in the graph. Because R^2 values > 0.7 are statically valid in such controlled experiments.

Table 6 : 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 globulus	Male	Young	46.1	0.7299	y = -0.97x + 127.75
			Adult	40.97	0.6132	y = -1.268x + 178.95
		Female	Young	66.6	0.713	y = -0.975x + 173.44
			Adult	33.1	0.6706	y = -6.225x + 666.65
4	Drusban (48%))	Male	Young	47.32	0.788	y = -1.2096x + 159.21
			Adult	114.58!	0.4783	y = -0.417x + 126.78
		Female	Young	55.86	0.7182	y = -1.286x + 203.34
			Adult	184.39!	0.1847	y = -0.3144x + 163.4
5	Malathion(48%)	Male	Young	70.28	0.6997	y = -0.9894x + 162.51
			Adult	60.45	0.7167	y = -1.290x + 205.98
		Female	Young	65.02	0.8906	y = -1.627x + 232.79
			Adult	76.23	0.8787	y = -1.6545x + 261.6
6	Ventazion /Diainon(60%)	Male	Young	59.04	0.885	y = -1.5534x + 215.7
			Adult	72.03	0.6958	y = -1.204x + 221.23
		Female	Young	57.82	0.7633	y = -1.8349x + 274.6
			Adult	70.37	0.8284	y = -1.692x + 283.08

e) Present inhibition of cochineal insect on spray

Considering the loss of aroma of the oils in an open environment 100-600μl oils were sprayed to each of the experimental cladodes for three days. As shown in the figure below (Fig. 4.1.) the results of all treatment groups are promising. Compared to the laboratory findings the less effectiveness (taking the three days in to account) of the treatment groups can be explained in many ways. The female attaches to the cladodes by their moths and the oils may not effectively get their heads until they blown or diluted by the air. The cladodes contain more than 200 insects of all ages. During each spray all of the species may not receive the treatment. The oil is too much volatile. And probably the insects may die quickly when they are starved. In literature of the carmine die producing company it was revealed that they are environment sensitive (Weniger1991).The environment of the laboratory could have contribution to the effect here.

i. *Percent of inhibition*

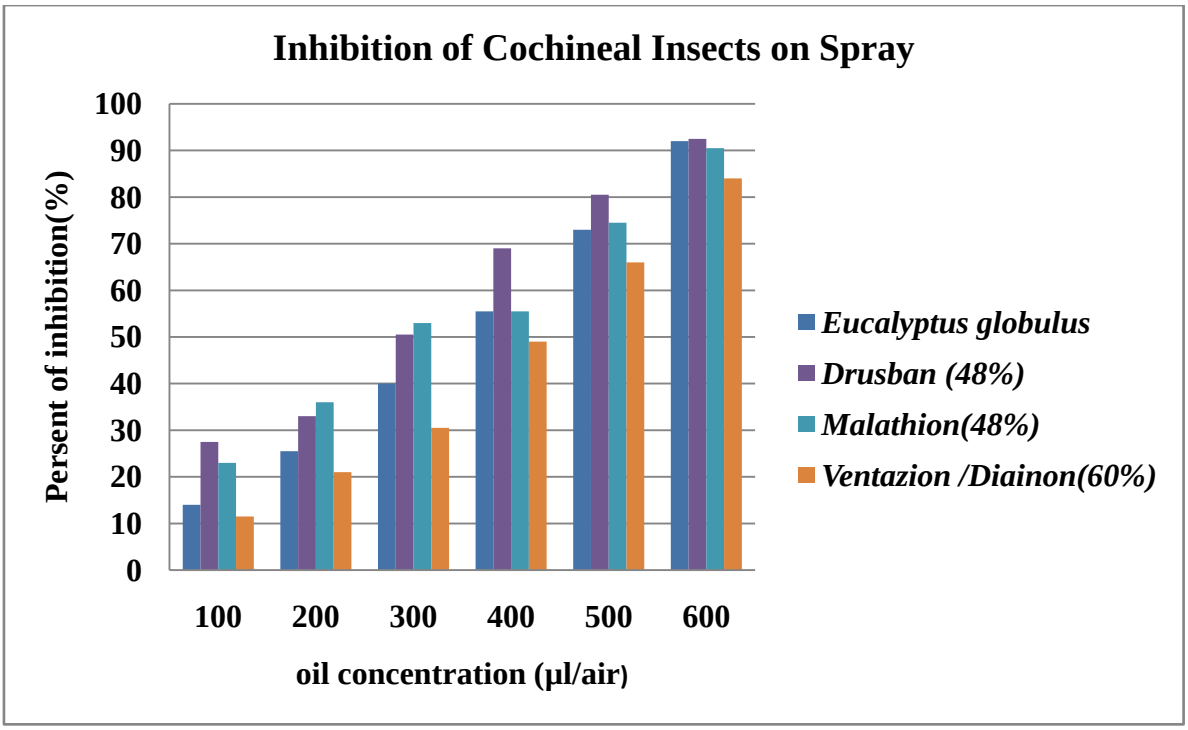

Fig. 1 : Inhibition of Cochineal Insects on Spray

ii. *Lethal concentration (LD50)*

Table 7 : Lethal concentration (LD50), Equation and R^2 values of treatment groups on spray

S/N	Treatment groups	LD50	R^2	Equation
1	*Eucalyptus globulus*	349.936	0.9932	y = 0.1566x - 4.8
4	Drusban (48%)	286.299	0.9852	y = 0.1389x + 10.233
5	Malathion(48%)	308.4804	0.9786	y = 0.1301x + 9.8667
6	Ventazion /Diainon(60%)	393.03	0.9814	y = 0.1474x - 7.9333

IV. Conclusion

If a plant possesses an essential oil it is believed by local peoples that it is medicinal plant. The aroma character of most of the plants used by traditional healers confirms this idea. Most insects are sensitive to plant aroma that they will be either attracted or repelled. Eucalyptus oils have strong aroma and irritating character capable of repelling the insects. The composite chemical nature of the oil is another big matter. The plants have many of these phytochemicals known for their inhibition towards microorganisms, insects and other foreign invaders. In addition, the oils tend to contain organic constituents, including hormones, vitamins and other natural elements with their own major role in the inhibition. This all could be the reason for the promising activity of the oils. In the in vitro assay in almost each jar the inhibition was dose dependent. Reasonable data was also recorded with respect to age and sex. A field test of course not in a big area as recommended by research works on insecticide was also done in six cladodes at six different concentrations. Elimination of the males both young and adult was very clear in three days of spray. Young female were also seen while getting dries out. However, the adult females are not mobile; their very small heads is mostly suck to the cladode that make it difficult to conclude whether or not they are lifeless. In general, it can be concluded that the oils from this variety of eucalyptus can repel and eliminate the cochineal insects. If the males of any age are eliminated the number of the colony will be too much decreased. There is no male insect means there is no way the insect will lay its 250 eggs in each cycle. There is no young female also means there is no adult female. Hence the adult female can be out of concern.

References Références Referencias

1. Buchbauer, G 2010, 'Biological properties of essential oils – an update' *Journal of Natural Products,* 4, 6-7.
2. Taylor and Francis Group, (2010), Handbook of essential oils Science, Technology, and Applications, Taylor & Francis Group New York 557-560.
3. Cipolca, L 2005, 'Chemistry of natural product compounds' *Organic and biomolecularchemistiry*, 3, 2-3.
4. Celikel, N and Kavas, G2008, 'Antimicrobial Properties of Some Essential Oils against Some Pathogenic Microorganisms' Czech Journal of Food Science, 6, 3, 174–181.
5. Derwich, E,Benziane, Z, Taouil, R, Senhaji, O, Touzani, M 2010,'Comparative Essential oil Composition of Leaves of Mentharotundifolia and Menthapulegium a Traditional Herbal Medicine in Morocco' *American European Journal of Sustainable Agriculture*, 4, 47-54.
6. Isman, M B 2000, 'Plant essential oils for pest and disease management' Crop Protection, 19,603-608.
7. Hussain, A2009, 'Characterization and biological activities of essential oils of some species of lamiaceae'*PhD thesis, University of Agriculture, Faisalabad.*
8. Can Baser, K H and Buchbauer, G 2010, 'Essential Oils Science, Technology, and Applications' Taylor & Francis Group, USA.
9. Mousa, K M, Khodeir, I A, El-Dakhakhni, T N and YoussefA E,2013, 'Effect of Garlic and Eucalyptus oils in comparison to Organophosphat insecticides against some Piercing-Sucking Faba bean insect Pests and natural enemies populations' *Academic Journal of Biological Sciences,* 5, 21 -27.
10. Yang, Y C, Lee, H S, Clark, J M and Ahn, Y J2004,'Insecticidal Activity of Plant Essential Oils Against Pediculushumanuscapitis (Anoplura: Pediculidae)'*Journal of Medicinal Entomologist*, 41, 700-704.
11. 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 MA2010, 'Chemical composition of Eucalyptus spp. essential oils and their insecticidal effects on Lutzomyialongipalpis' *VetParasitol*, 167, 1–7.
12. Gebretsadik, G, Animut, G, Tegegne, F2013, 'Assessment of the potential of cactus pear (Opuntiaficusindica) as livestock feed in Northern Ethiopia' Livestock Research for Rural Development, 25 (2),Retrieved August 26, 2015, from http://www.lrrd.org/lrrd25/2/moen25026.htm
13. Saim, N, Osman, R, Hirmi M D,Yasin, A and Hamid,R 2008, 'Subcritical water extraction of essential oil from coriander (Coriandrumsativum L.) seeds' The Malaysian Journal of Analytical Sciences, 12, 1-2.

14. Fabiane, K, Ferronatto, R, Santos, A and Onofre, B2007, 'Physicochemical characteristics of the essential oil of Baccharisdracunculifolia and Baccharisuncinella D.C. (Asteraceae)' Journal of. Pharmacognosy, 18,197-203.
15. Tiwari, P, Kumar, B, Kaur, M, Kaur, G and Kaur, H 2011, 'Phytochemical screening and Extraction: A Review' internationalepharmaceuticasciencia, 1,103-104.
16. Jeyachandran, R, Mahesh, A, Cindrella, L, Sudhakar, S and Pazhanichamy, K2009, 'Antibacterial Activity of Plumbagin and Root Extracts of Plumbago Zeylanica L.' *Seri. Botanica*, 51, 17–22.
17. Kashiwada, Y 1992, 'Antimouragents, Tannins and related compounds as selective cytoxic agents, Journal of Natural Products, 55, 1033-1043.
18. 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, MemInst Oswaldo Cruz, 98, 959-961.
19. Jagessar, C R and Cox, M2010, 'Phytochemical Screening of the CHCL3 and CH3CH2OH Extract of Stems, Twigs, Roots and Barks of Conocarpus Erectus. L.' International Journal of Academic Research, 2, 36-45.
20. Schulz, H, Baranska, M, Belz, HH, Ro¨sch, P, Strehle, M A and Popp, J 2004, 'Chemotaxonomic characterisation of essential oil plants by vibrational spectroscopy measurements' Vibrational Spectroscopy, 35, 81–86.
21. Weniger, D. 1991. Cacti of Texas. The University of Texas Press,