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## Essential Plant Oils and Insecticidal Activity in *Culex quinquefasciatus*

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### 1. Introduction

Plants have great importance for man because they are one of their sources of food, they provide us through the process of photosynthesis the oxygen we breathe and are essential to maintain the ecological balance (Corbino, 2000).

Essential oils are volatile, usually distillable liquid fractions responsible for the aroma of the plant. The vast majority of them are pleasant smell and its metabolic and evolutionary significance lies in the role they play as attractor of pollinating agents (for its pleasant aroma), constitute elements of defense against the attack of parasites, herbivorous animals and insects, allow the adaptation of the plant when water is scarce and are part of the substances in reserve as the giver of H<sup>+</sup> in the processes of electron. The organoleptic characteristics of the essential oils may be given by major components, although in other cases they are substances present in tiny quantities (traces) which define the taste, smell, or therapeutic properties (Scholes, 1995; Worwood, 1992).

These natural substances are known as secondary metabolites, name which refers to substances that are not involved in the basic mechanisms of life of the plant but that comply with specific functions. (Corbino, 2000). They tend to accumulate in large amounts without negative effects and represent a problem in the cell or on the plant. These metabolites have the property to form glycosides and are found soluble in the plant. For many years these metabolites were regarded as final products of metabolic processes without specific function or directly as a waste of plant products (Lopez, 2008).

The study of these substances was initiated by organic chemists of the 19th century and early 20th century who were interested in these substances because of its importance in the medical industry, the manufacture of flavoring, etc. In fact, the study of the secondary metabolites stimulated the development of separating techniques, and spectroscopy for determining their structure and synthesis which constituted the basis of the contemporary organic chemistry (Lopez, 2008).

They can be found in different parts of the plant: leaves (wormwood, basil, buchú, cidrón, eucalyptus, mint, lemongrass, marjoram, mint, patchouli, quenopod, rosemary, Sage, lemon balm, etc.), in the roots (angelic, asaro, saffron, calamus, turmeric, galanga, ginger, sandalwood, Sasafras, Valerian, vetiver, etc.), in the pericarp of the fruit (lemon, Tangerine, Orange, etc.), seeds (anise, cardamom, dill, fennel, cumin, etc.), in the stem (cinnamon, caparrapí, etc.), flowers (arnica, lavender, chamomile, pyrethrum, thyme, clove scent, rose,

etc.) and in the fruit (caraway, coriander, bay leaf, nutmeg, parsley, pepper, etc.). ([www.buscasalud.com](http://www.buscasalud.com))

## 2. Essential oils

Essential oils are complex mixtures of up to over 100 secondary metabolites, which can be classified in terpenoid compounds, phenolic and alkaloids based on their biosynthetic origins. Terpenes containing a single unit of isoprene are called monoterpenes, which contain three units named sesquiterpenes (Ikan, 1991; Silva, 2002).

Monoterpenes and sesquiterpenes are terpenes from 10 to 15 atoms of carbons biosynthetically derived from geranylpyrophosphates (SPG) and farnesilpyrophosphate (FPP) respectively. Monoterpenes and in general all the natural terpenoid compounds are synthesized by the route of the acetylCoA through a common intermediate which is the mevalonic acid. However, it has been proposed that some terpenoids are not originated by this route, and instead use an alternative route that may involve pyruvate, glyceraldehyde-3-phosphate (Fig 1) (Adams et al, 1998; Sponsel, 1995).

Essential oils are widely distributed in some 60 families of plants including the Compositae, Labiadas, Lauraceae, Myrtaceae, Rosaceae, Rutaceae, Umbelliferae, etc. Monoterpenoides are mainly found in plants in the family of Ranunculales, Violales, and Primulales, while they are rare in Rutales, Cornales, Lamiales, and Asterales. Moreover, the sesquiterpenoides are commonly found in Rutales, Cornales, Magnoliales and Asterales. Although in both essential oils: mono - sesquiterpenes and phenylpropan are found in free-form, those which are linked to carbohydrates, have been more recently investigated, because it is considered to be the immediate precursor of the essential oil.

From the chemical point of view, despite its complex composition with different types of substances, essential oils can be classified according to the type of substances of which they are the major components. According to this fact, the essential oils rich in monoterpenes are called essential oils sesquiterpenoids, those rich in phenylpropan are the essential oils phenylpropanoids. Although this classification is very general there are more complex classifications that take into account other chemical aspects (Fig 2,3,4) (González, 1984; Ikan, 1991; Judd et al, 2002; Stachenko, 1996).

### 2.1 Methods of extraction

According to [www.chemkeys.com](http://www.chemkeys.com) 2000; Gascon et al. 2002; [www.herbotecnia.com.ar](http://www.herbotecnia.com.ar), 2002; [www.losandes.com.ar](http://www.losandes.com.ar) 2002. essential oils can be extracted from plant samples through various methods samples such as: expression, distillation with steam, extraction with volatile solvents, enfleurage, extraction with supercritical fluids and hydrodistillation.

### 2.2 Method of isolation

From such oils, it is possible to its isolation through the use of one or several chromatographic methods such as thin layer chromatography and HPLC.

For the chromatographic column and thin layer methods the silica gel are widely used as stationary phase. Polar pure or mixed solvents such as: Toluene-acetate of ethyl benzene, chloroform, dichloromethane, ethyl, chloroform-benzene, chloroform-ethanol - chloroform-benzene acetic acid benzene-acetate are used as mobile phase.

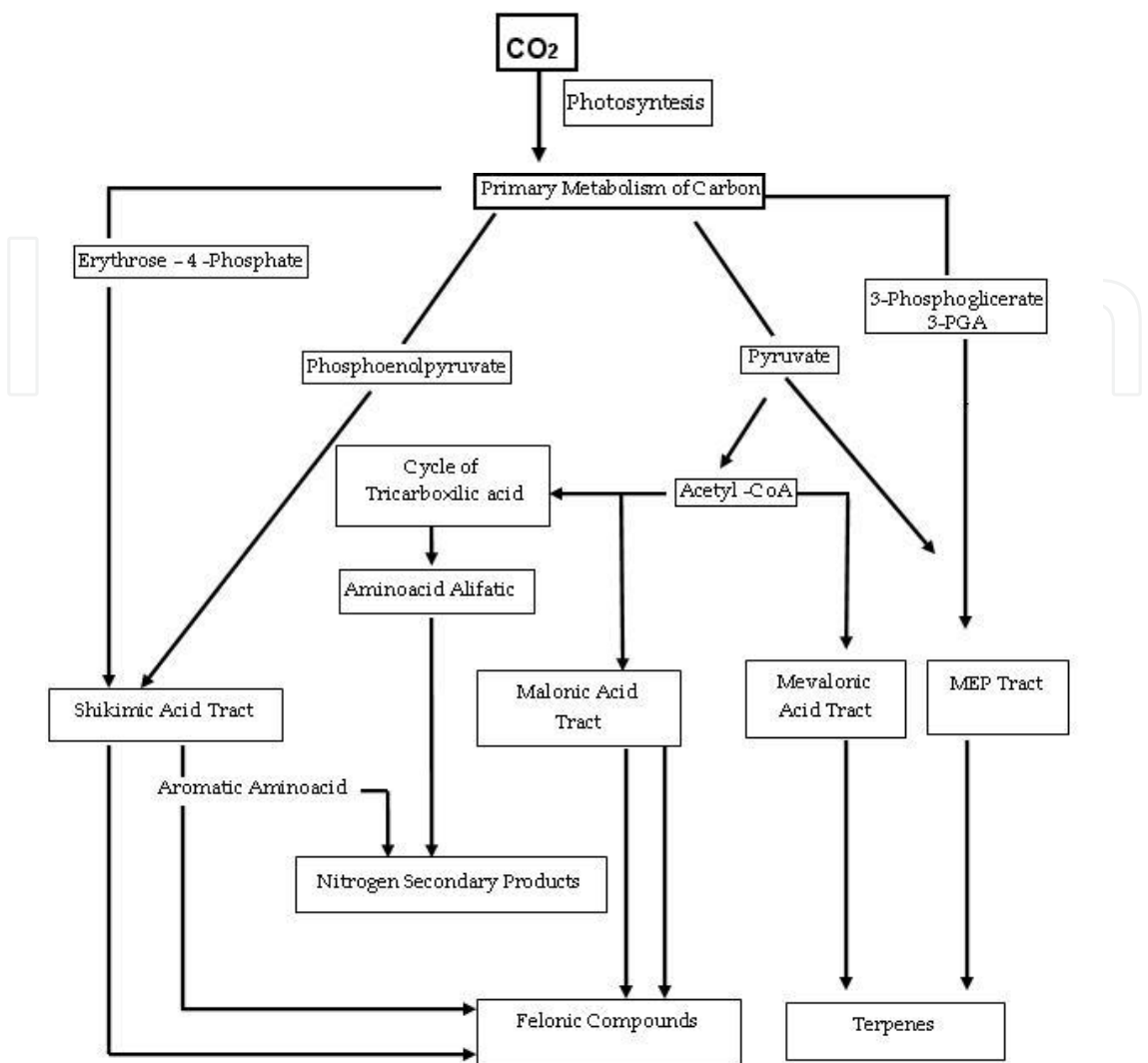


Fig. 1. General pathways of plant secondary metabolism.

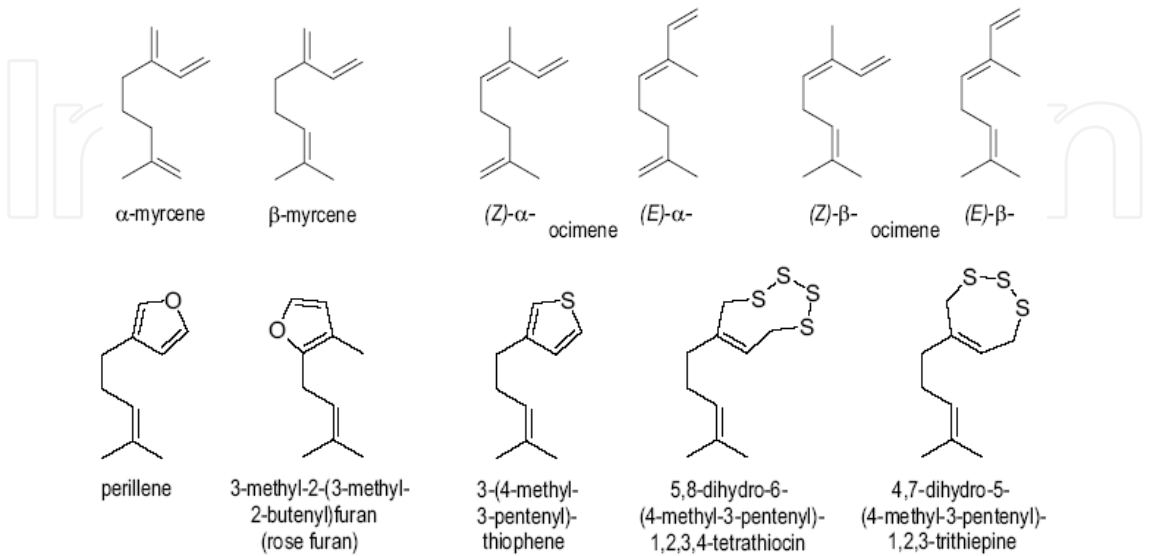


Fig. 2. Natural Monoterpenes.

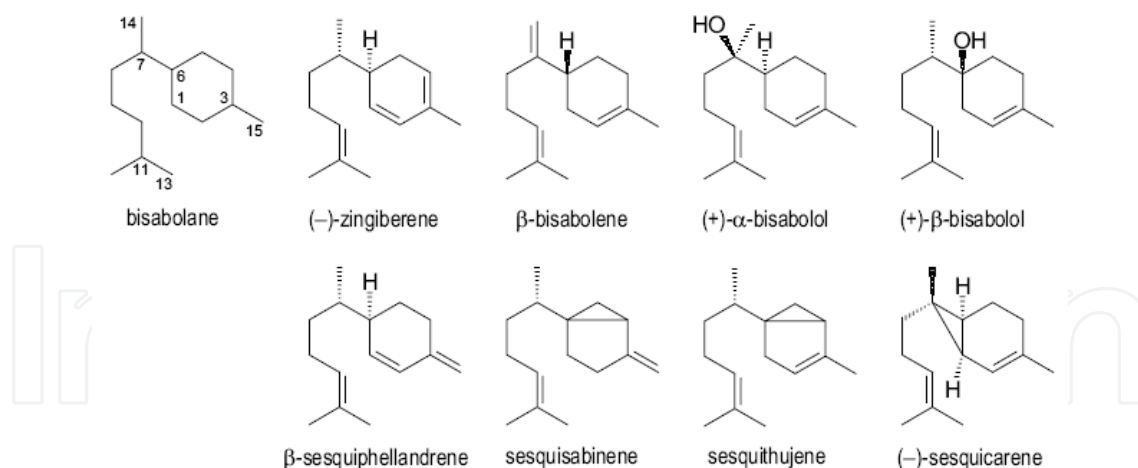


Fig. 3. Natural Sesquiterpenes.

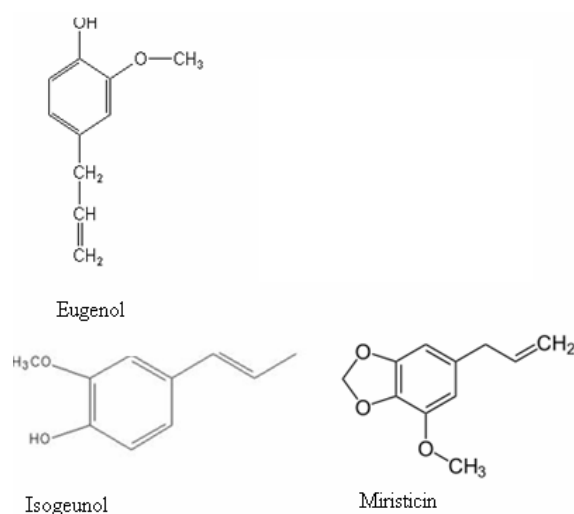


Fig. 4. Natural phenylpropanoids.

However, currently more efficient and faster techniques of separation are used such as HPLC high efficiency liquid chromatography and gas (GC) chromatography, as well as also combinations "ON-LINE" HPLC- GC EM. These same methods are used for the analysis of the flower essences. The last technique, thanks to the recent development of capillary columns for high resolution, allows analyzing complex mixtures present in essential oils, and identify the components from the retention times through the so-called Kovats retention indexes (Ik). These values are characteristic for each component and there are databases with indexes of many components of essential oils. Ik values are determined in two chromatographic columns, one polar (e.g. CARBOWAX 20 M) and a non-polar (e.g. OV - 101 also called DB-1).

In addition, the docked technique chromatography of gases - mass spectrometry, (GC-MS) allows obtaining the spectrum of mass of each component which builds the molecular weight and structural information. Likewise there are databases with spectra of masses of many components, something similar goes for the index Kovats (determined in two columns of different polarity) and the spectra of mass are criteria for chemical allocation of many components of essential oils not just monoterpenes but also other types of substances characteristic of such oils. More recently the column chromatographic Chiral for separation

of optically active components have been developed, and have developed methods for combined analysis HPLC HPLC-NMR of mixtures of sesquiterpenes and mass spectrometry.

### 2.3 Test of recognition of monoterpenes and sesquiterpenes (Pino, 1999; Stam, 1970)

Due to the diversity of functional groups that can be present in the components of mono- and sesquiterpenes of an essential oil, there is a specific test for their recognition. However there are few experimental procedures that allow to recognize some of them by their coloration with different reagents, its absorption of 254 nm UV light and its  $R_f$  in thin layer chromatography. For example, limonene is recognized in the plates of TLC because it does not absorb UV 254 nm light, adds bromine, does not form a derivative 2, 4-dinitrophenylhydrazone and produces Brown with sulfuric acid.

There is a procedure which combines the TLC with micro reactions (oxidation, reduction, dehydration, hydrolysis, etc.) described in the classic book already today of Ikan. Other reagents useful for revealing monoterpenes and sesquiterpenes are anisaldehyd-sulfuric acid, sulfuric acid -vanillin and phosphomolibdic acid.

#### 2.3.1 Spectral characterization

Monoterpenes and sesquiterpenes, in a number, can be characterized chemically from the gas chromatography and mass spectra data as noted above, but when there are doubts of such characterization some spectral methods such as infrared ultraviolet and magnetic resonance spectrometry can be used.

##### 2.3.1.1 Infrared spectroscopy

Infrared Spectroscopy allows detection of the presence of groups hydroxyl, Carbonyl, aromatic rings, bond double  $C = C$  cis and trans, etc. To determine the spectrum just put a drop of the component in a cell of NaCl. For example, in the infrared spectrum of the 3-p-menten-7 present in the oil of cumin, the intense band in  $1725\text{ cm}^{-1}$  indicates a group not conjugated Carbonyl. The peak at  $2710\text{ cm}^{-1}$  is assigned to a proton aldehydic C-H. The bond around on  $1375\text{ cm}^{-1}$  indicates a group isopropyl, and the band of  $817\text{ cm}^{-1}$  average intensity indicates a double bond tri-substituted.

##### 2.3.1.2 Ultraviolet spectroscopy

UV spectrum ultraviolet of monoterpenes and sesquiterpenes allows the recognition of functional groups and chromophoric. E.g. limonene presents an absorption maximum at 262 nm ( $\xi = 6400$ ).

Nuclear magnetic resonance ( $^1\text{H} - ^{13}\text{C}$ ); due to the developments of the NMR has database of Spectra, especially of  $^{13}\text{C}$  -NMR for the monoterpenes and more distributed sesquiterpenes. The NMR spectrometry- $^{13}\text{C}$  has the additional advantage that the carbons of terpenoid chemical displacement (and other natural and synthetic substances) can be calculated by computer programs available in the market as Chemwind and ACD-Lab.

In addition, the recent development of two-dimensional methods homo- and heteronuclear, has allowed the fine structural determination of terpenoids and other natural substances, eliminating the ambiguity in the allocation of the observed signals.

##### 2.3.1.3 Mass Spectrometry

There are databases that contain information and spectra of masses of the components of mono- and sesquiterpenoids of essential oils. These databases are now available in many



commercial instruments of analysis as a chromatograph of gases coupled with mass spectrometers.

## 2.4 Phenylpropan

Phenylpropanes are secondary metabolites derived from phenylalanine, which is first converted to cinnamic acid and after a series of hydroxylations lead to acid cumaric, caffeic acid, pterulic acid etc. The conversion of these acids to their corresponding esters produces some of the volatile components responsible for the fragrance of herbs and flowers (Lopez, 2008).

Phenylpropanes of essential oils are extracted with the same methodology described above for mono - and sesquiterpenes. However, due to its aromatic ring they have advantages in detection by TLC and HPLC because absorb ultraviolet light (254 nm) and do not require to be disclosed with chemical agents, or need to be derivative, and therefore can be isolated and analyzed more easily.

### 2.4.1 Recognition of phenylpropan

There are test assay for recognition to the aromatic ring as a reaction with formaldehyde and sulfuric acid. Likewise in the case of phenylpropan with phenolic hydroxyl these can be recognized by the test assay of ferric chloride, which produces green and blue coloring with phenolic substances in general.

Substances with aromatic ring, their infrared spectra show signs characteristic of these compounds and give information about the type of replacement of the aromatic ring in addition to the functional groups present in the molecule. For example, the spectrum of eugenol shows, among other, bands in 3500 (wide) due to the hydroxyl group, 1510 characteristic of aromatic, and three bands in 990, 920 and 938  $\text{cm}^{-1}$  characteristics of a vinyl mono substituted group. The IR from the cinamaldehyde spectrum displays bands in (weak) 3330, 3050, 2820, 2750, 1660 (intense, due to the carbonyl group), 975, 740 and 695  $\text{cm}^{-1}$  among others.

Unlike the majority of mono - and sesquiterpenes, the phenylpropanes absorb UV light with a maximum around 254 nm depending on the groups present in the molecule chromophors. For example, the isoeugenol shows maxima at 260 (15850) and 305 (7000), safrole in 286 nm, the myristicine at 276 nm, the isosafrole in 264 nm, trans- cinnamic acid in 273 nm and cis- cinnamic acid in 264 nm.

Phenylpropan  $^1\text{H}$ -NMR spectra show signs of aromatic protons around 6-8 ppm whose multiplicities and coupling constants allow a clear structural assignment even with low resolution spectra. Trans-anethole there is a double signal around 1.9 ppm due to the protons from the methyl group, a singlet in 3.9 due to the protons of the methoxy group, a complex signal around 6.1 ppm due to the two protons olefinics provision trans each other, and a double around 6.9 ppm characteristic of 4 protons of an aromatic ring p-based.

## 2.5 Natural insecticides way-of-action (Silva 2002)

As growth regulators, this effect can manifest itself in several ways. The first is related to the molecules that inhibit the metamorphosis. Other compounds make the insect to have an earlier metamorphosis, occurring at a time which is not favorable. It has been observed that certain molecules can alter the function of the hormones that regulate these mechanisms so that there are insects with malformations, dead or sterile (Gunderson, 1985; Sangykurn, 1999).

Studies carried out from different concentrations of extract of Meliaceas show that this extract inhibits the feeding and negatively affects the development and survival of different species of insects. The anti-alimentary activity of this compound shows that at doses ranging from 5.5 to 27.6  $\mu\text{g}/\text{cm}^2$  caused an inhibitory activity of more than 75% the insects. The way of action of these compounds extracted from various species of Meliaceas are taken from a combination between an anti feeding effect and post-digestive toxicity. (www.cannabiscultura.com)

The use of plants as repellents is an ancient knowledge. This practice is basically done with compounds that have odor or irritant effects such as chili and garlic. There are homemade recipes that describe the use of fennel (*Foeniculum vulgare*), rue (*Ruta graveolens*) and eucalyptus (*Eucalyptus globules*) among other aromatic plants to repel moths of clothing.

### 2.5.1 Mode of action of the natural insecticide

Treatments with natural compounds such as essential oils or pure compounds (Awde & Ryan, 1992; Keane & Ryan, 1999; Ryan & Byrne, 1988) may cause symptoms that indicate neurotoxic activity including hyperactivity, seizures and tremors followed by paralysis (knock down), which are very similar to those produced by the insecticides pyrethroids (Kostyukovsky et al., 2002).

Acetyl cholinesterase enzyme catalyzes the hydrolysis of the neurotransmitter excess acetylcholine in the synaptic space between choline and acetic acid. It has been recognized that essential oils could their effect through ACE inhibition.

Many hydrophobic compounds incite the deactivation of protein and enzyme inhibition; the acetylcholinesterase (AChE) is an enzyme that is particularly susceptible to the hydrophobic inhibition (Ryan et al. 1992).

Is described in this context essential oil interfering with AChE are acting as potent of the central nervous system where all synapses cholinergic are virtually located (Bloomquist, 1999) of the cholinesterase inhibitors are known as anticholinesterasic, the chemical products that interfere with the action of this enzyme are potent neurotoxins (López, 2008).

The majority of plant insecticide are extracts made by a group of active compounds of diverse chemical nature, which is hardly found in the same concentrations so the pressure of selection on the plague will not be always the same, i.e. in general, insects take more to develop resistance to a blend of compounds than any of its components separately (Silva, 2003).

### 2.6 Future prospects

With the beginning of the new millennium, assessment of a large number of essential oils from native or typical flora has increased in many countries of the Americas and Africa due to its potential use as an alternative method of control.

There have been studies with oils in laboratory conditions reporting larvicide action in species of the genus *Culex*, *Anopheles*, *Aedes* (Ansari, 2005; Assarn, 2003; Albuquerque et al, 2004; Amer et al, 2006a ; Bassole et al., 2003; Carvalho et to 2003; Cavalcanti et al, 2004; Cetin et al, 2006; Cheng et to 2004; Chung et 2010 b Dharmagda et al, 2005; of Mendonca et al., 2005; Faley et al., 2005; Hafeez et 2011; Khandagle et al, 2001; Mathew & Thoppil 2011;Morais et al, 2006; Prajapati et al., 2005; Pushpanathan et al, 2006; Phasomkusolsil & Soowera 2010; Pitarokili et al, 2011; Raví et al, 2006; Tare et al, 2004; Tomas et al, 2004).

In the same way it has been evaluated the repellent capacity of some oils in the same species of mosquitoes; Amer et al, 2006b; Barnard et al, 2004; Byeoung-Soo, 2005; Caballero-



Gallardo, et-2011; Chang et al, 2006; Jaenson et al, 2006; Das et al., 2003; Kim et al, 2004; Oyedale et al, 2002; Odalo et al., 2005; Pohilt et 2011; Phasomkusolsil & Soowera 2010; Tawatsin et al, 2006; Trongtokit et al. 2005a; 2005b; Yang et al., 2005; Zhu et al, 2006; Less has been evaluated adulticide effect found only two reports described by Chaityasit et al 2006 and Miot et al 2004.

### 3. Studies developing in Cuba with essential oils for controlling of public health pests

In Cuba there is a large plant biodiversity with a lot of endemic species. Myrtaceas, Piperaceas, Zingiberaceae, Pinaceae families possess insecticidal action potential based to their essential oils. To this day, there have been verified in public health pests such as; *Blatella germanica*, *Musca domestica* and *Aedes aegypti* (Aguilera et al 2003; 2004; Leyva et al, 2007 a, b, 2008a, b; 2009a, b; 2010) and more recently in *Culex quinquefasciatus*.

Evaluated essential oils are essentially from *Pimenta racemosa*, *Eugenia melanadenia*, *Psidium rotundatum* and *Melaleuca leucadendron* of the botanical family Myrtaceae, *Piper auritum*, *Piper aduncum* of the family Piperaceae, *Curcuma longa* in the family Zingiberaceae, *Artemisia abrotamum* in the family Asteraceae, *Pinus tropicalis* and *Pinus caribbaea* of the Pinaceae family. A derivative of essential oil of pinaceas has also been evaluated; turpentine oil obtained by hidrodestylation of the resin of endemic pine trees, mostly composed of terpenes ( $\alpha$ - and  $\beta$ -pinene and a modified turpentine where a part of the original turpentine oil was modified by photoisomerization of the  $\alpha$  and  $\beta$ -pinene to compound of the type verbenone and pulegone.) In all tested oils, there has been found necessary dosages that elicited from 5% to 95% of mortality. After testing CHI2 they have a probability greater than 0.05 by which can raise all the mortalities occurring in each are associated with the used dose.

Studies with these oils in larvae of *Aedes aegypti* show high insecticidal action by the low lethal concentrations obtained and the high slopes of the regression lines; however, in *Blatella germanica* and *Musca domestica* the bioassays were conducted in adults, showing a very low activity given the high lethal concentrations obtained.

Natural essences of plants owe its insecticide and repellent action to the presence in its composition of derivative monoterpenic as d-limonene,  $\alpha$ -terpineol,  $\beta$ -myrcene, linolool, 1,8-cineole, 4-terpineol, thymol, carvacrol,  $\alpha$ -pinene and  $\beta$ -pinene.) Studied oils possess structures lactones as the 1,8 cineole, 4-terpinol,  $\alpha$  and  $\beta$ -pinene, cineole, safrole, turmenon, chavicol, eugenol, beta-phellandrene, longifoleno among others, which account for a 18-68% of its total membership, and are that they can be attributed the high insecticidal action in *Aedes aegypti*.

One aspect assessed for reasons of eco-sustainability was ovicidal and inhibitory action of the development by the oil of turpentine in larvae of *Ae aegypti*

The largest ovicidal effect was shown with the dose diagnosis of oil of turpentine with photochemical treatment. In a test of hypothesis of different proportions for each dose of oil, the diagnostic doses were compared with every  $CL_{95}$  finding differences between the doses tested with modified oil of turpentine for  $p=0.05$  while for oil not modified there was only significant difference comparing the  $CL_{95}$  and the diagnostic doses. ( $p=0.05$ ). By comparing the  $CL_{95}$  and the diagnostic dose, he found significant difference for a  $p = 0.05$ . If we compare both types of essences we may conclude that although modified turpentine oil presents a  $CL_{95}$  dose higher than oil without changing this it has a greater effect ovicidal and therefore more protector.

The percentage of hatching inhibition was 36.47% of the total number of larvae. The larvae completing their development to adults corresponded to 60.54% of surviving larvae. As a figure of interest the greatest number of semi emergency in adults occurred at 72 hours after exposure to the oil of turpentine (with a predominance of males). At 96 hour, the largest proportion was in favor of females, who became non-existent for this sex at 120 and 168 hours (5-7 d)

More recently, oil of turpentine and the bicyclic  $\alpha$ -pinene obtained by fractional distillation of oil of turpentine have been evaluated in *Culex quinquefasciatus*, important vector of West Nile virus and encephalitis

The purification of the  $\alpha$ -pinene was made using column chromatography in system of variable stationary phase ( $\text{SiO}_2$ ,  $\text{Al}_2\text{O}_3$ , and their mixtures in dependence of the polarities of the mobile phases for elution and separation.) Molecular characterization was developed, using techniques of FTIR and NMR, as well as methodologies for its extension to field conditions type TLC (chromatography on thin layer in the presence of plates of glass with silica gel 200-G254 activated with  $\text{AgNO}_3$  / acetonitril via wet impregnation with methanol). The plates are eluted with mixtures of solvents of media polarity (ethyl-acetone - alcohol isopropyl acetate). Figure 5 shows the spectroscopic characterization of the  $\alpha$  pinene. In table 1 and 2 is shown the concentrations finally used, the obtained mortalities and lethal concentrations that causes 50% to 95% percent of mortality, the slope of the regression line and diagnostic dose.

The lowest lethal concentrations were obtained with oils of turpentine and turpentine modified followed by *Curcuma longa*, *Psidium rotundatum* and *Chenopodium ambrosioides*. By applying a  $\chi^2$ -square test ( $p < 0.05$ ) to the results of the bioassays, the results showed that the mortalities are associated with the used dose. The higher slopes of the lines were equal with the oil of turpentine, *Eugenia melanadenia*, *Psidium rotundatum* and *Chenopodium ambrosioides*, corresponding to the lower value of the slope to the terpene  $\alpha$ -pinene. To the slope values obtained were applied a  $\chi^2$  test finding significant difference between them for  $p < 0.0001$ , indicating that these oils have a different response for the same strain.

In our assessed results we find the insecticidal action of oil in *Cx quinquefasciatus* and is important to note that lethal concentrations obtained are higher than concentrations found in *Aedes aegypti* to these same oils (Leyva, 2008a; 2009a,b). This response could be that this species has developed physiological resistance and some tolerance to insecticides with which it has active defense before any insecticide action.

Oil of *Curcuma longa* presents in its composition more than 50% of sesquiterpenes, apart from the monoterpenes and  $\alpha$ -pinene to which can be attributed the insecticide action. If we compare studies conducted with *Curcuma aromatica* (Choochate et al., 2005) and *Curcuma zedoaria* in *Aedes aegypti* these oils show lower values of  $\text{LC}_{50}$  and  $\text{CL}_{99}$  (Champakaew et al, 2007).

Within the family Piperaceae, notably members of the genus *Piper* have noted insecticidal action, molluscicide (Chansang et al., 2005; Parmar et al 1997) this is due to the presence of the secondary metabolites as alkaloids, phenylpropanoids, lignans, neolignans, terpenes, flavonoids, among others (Pino et al, 2004; Smith & Kassim 1979). *Piper aduncum* specifically shows in its composition a high percent of dilapiol,  $\alpha$ -pinene and 1,8- cineole (Bottia et al 2007). In Mexico studies were not mortalities obtained with a kind of *Piper* in *Culex quinquefasciatus* in aqueous extracts to 5 and 15% where used in the aerial part of plants (Perez-Pacheco et al 2004).

The larvicidal activity of *Chenopodium ambrosioides* was evaluated in *Ae aegypti*, with extracts metanolics to different concentrations resulting most important inhibition of pupal development and an increase in the time of larval development (Suparvarn et al 1974) has also been evaluated in mosquito repellent activity (Gillij et al 2008). *Ch ambrosioides* has as one of its components majority the carvacrol reported in the literature as insecticide terpene (Silva et al 2008; Kordali et al 2008). *Pimenta racemosa*, *Eugenia melanadenia* and *Psidium rotundatum* presented in its composition more than 50% of monoterpenes and sesquiterpenes standing out in his majority composition 1,8 cineole, 4-terpineole and  $\alpha$ -pinene terpenes with high insecticidal action (Pino et al, 2004,2005). Oil of turpentine in *Aedes aegypti* obtained strong insecticide and inhibitory action of development in previous studies (Lucia et al, 2007; Leyva et al, 2009b 2010). In this study isolated terpene  $\alpha$ -pinene (one of the major components of turpentine) was higher than the own turpentine lethal concentrations, this may be due to the synergistic effect with several monoterpenes ( $\alpha$  and  $\beta$ -pinene) at the same time and not isolated and evaluated by themselves in insects.

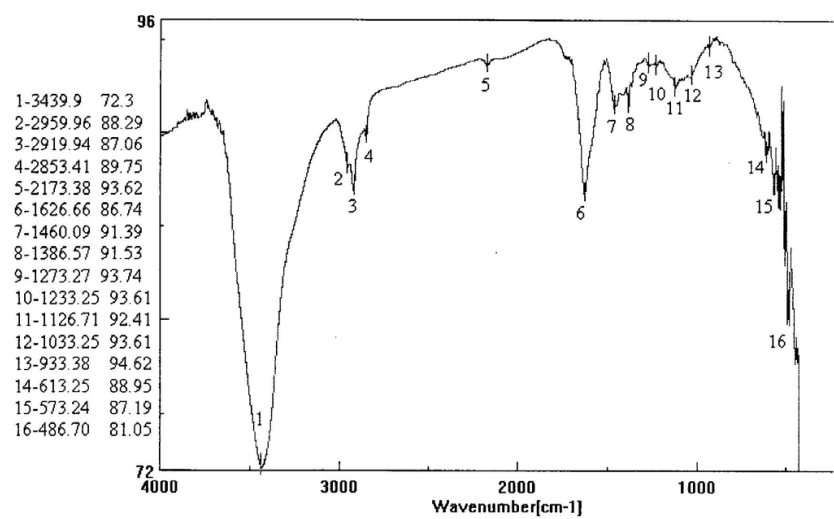


Fig. 5. Spectroscopic characterization of the  $\alpha$  pinene.

Conc (%)	Mortality (%)	Lethal Conc		Slope of the Line
Turpentine				
0.0035	5	CL <sub>50</sub>	0.0042	20.9
0.0040	31			Diagnostic
0.0050	94	CL <sub>95</sub>	0.0055	Dose
0.0060	100			0.0108 (108 mg/L)
Modified Turpentine				
0.0030	5	CL <sub>50</sub>	0.0038	14.4
0.0040	60			Diagnostic
0.0050	93	CL <sub>95</sub>	0.0050	Dose
0.0060	100			0.0112 (112 mg/L)

$\alpha$ -pinene				
0.0040	35	CL <sub>50</sub>	0.0052	3.7 Diagnostic Dose 0.044 (440 mg/L)
0.0050	46			
0.0060	56	CL <sub>95</sub>	0.014	
0.0070	67			
0.0080	78			

Table 1. Concentrations used and rates obtained with derivatives of Pinaceae in *Cx quinquefasciatus* larvae.

Conc (%)	Mortality (%)	Lethal Conc		Slope of the Line
Chenopodium ambrosoide				
0.0040	10			11.8 Diagnostic Dose : 0.0162 (162 mg/L)
0.0050	42	CL <sub>50</sub>	0.0051	
0.0060	80			
0.0070	95	CL <sub>95</sub>	0.0071	
0.0080	98			
Curcuma longa				
0.0040	46			5.22 Diagnostic Dose : 0.0232 (232 mg/L)
0.0050	66	CL <sub>50</sub>	0.0041	
0.0060	80			
0.0070	86	CL <sub>95</sub>	0.0086	
0.0080	94			
Psidium rotundatum				
0.0040	28			8.16 Diagnostic Dose 0.018 (180 mg/L)
0.0050	58	CL <sub>50</sub>	0.0047	
0.0060	80			
0.0070	92	CL <sub>95</sub>	0.007	
0.0080	97			
Piper aduncum				
0.0100	36			6.72 Diagnostic Dose 0.050 (500 mg/L)
0.0120	53	CL <sub>50</sub>	0.0110	
0.0140	73			
0.0160	85	CL <sub>95</sub>	0.0200	
0.0180	90			
0.0200	95			
Pimenta racemosa				
0.0040	10			5.23 Diagnostic Dose 0.0380 (380 mg/L)
0.0050	20	CL <sub>50</sub>	0.0070	
0.0060	44			
0.0070	50	CL <sub>95</sub>	0.0140	
0.0080	62			
0.0100	79			
Eugenia melanadenia				
0.0240	14			18.5 Diagnostic Dose 0.0718 (718 mg/L)
0.0260	41	CL <sub>50</sub>	0.026	
0.0280	70			
0.0300	78	CL <sub>95</sub>	0.033	
0.0320	90			

Table 2. Concentrations used and rates obtained with essential oils tested in *Cx quinquefasciatus* larvae.

#### 4. Conclusions

An interest in natural products from plants has been increased due to the development of resistance to synthetic insecticides, which are applied in order to reduce the populations of insects.

The biological activity of natural compounds is based on its structure and the doses used for such purposes. Vegetable insecticides have the great advantage of being compatible with other acceptable low-risk options from the ecological point of view on the control of insects. The secondary metabolites produced by plants against the attacks of predators and insects make them natural candidates in the control of species of insects, both vector of diseases and pests of agriculture. It is not logical to come to jump to the idea that they will completely replace the synthetic insecticides. Logical thinking is to have in them a complementary use to optimize and increase the sustainability of current integrated pest control strategies.

There are many publications of lists of plants with insecticidal properties. To use such plants, it is not enough to be regarded as promising or proven insecticidal properties. Analysis of risks to the environment and health should also be made. It is not to recommend the use of plants that are endangered, with limited biomass or that their use involves major alterations to the density in which they are in the nature. An ideal insecticide plant must be perennial, be widely distributed and in large amounts in nature or that can be cultivated, using renewable plant bodies such as leaves, flowers or fruits, not be destroyed every time you need to collect material to (avoid the use of roots and bark), agro-technician minimum requirements and be eco-sustainability, have additional uses (such as medicines), not having a high economic value, be effective at low doses, possess potential scaling biotechnology (Silva;2002).

Insecticide plants have the advantage of having other uses as medicinal, a rapid degradation which decreases the risk of residues in food and therefore can be more specific for pest insect and less aggressive with natural enemies. They also develop resistance more slowly in comparison with synthetic insecticides. By the other hand, the disadvantages include that they can be degraded more quickly by ultraviolet rays so its residual effect is low, however not all insecticides from plants are less toxic than synthetic and residual is not established.

We can conclude that studies in our country, essential oils and their derivatives from the different families of evaluated plants have a high insecticide activity on larvae of *Aedes aegypti* and *Culex quinquefasciatus* and derived specifically from pinaceas, they have ovicide and inhibitory action of development in *Aedes aegypti*, which are potential candidates for control alternatives in these species of insects.

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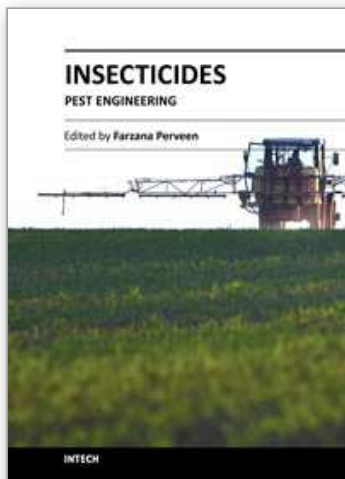
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## **Insecticides - Pest Engineering**

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This book is compiled of 24 Chapters divided into 4 Sections. Section A focuses on toxicity of organic and inorganic insecticides, organophosphorus insecticides, toxicity of fenitrothion and permethrin, and dichlorodiphenyltrichloroethane (DDT). Section B is dedicated to vector control using insecticides, biological control of mosquito larvae by *Bacillus thuringiensis*, metabolism of pyrethroids by mosquito cytochrome P40 susceptibility status of *Aedes aegypti*, etc. Section C describes bioactive natural products from sapindacea, management of potato pests, flower thrips, mango mealy bug, pear psylla, grapes pests, small fruit production, boll weevil and tsetse fly using insecticides. Section D provides information on insecticide resistance in natural population of malaria vector, role of *Anopheles gambiae* P450 cytochrome, genetic toxicological profile of carbofuran and pirimicarp carbamic insecticides, etc. The subject matter in this book should attract the reader's concern to support rational decisions regarding the use of pesticides.

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