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ARTICLE

Terpenes of the Essential Oil from *Ipomoea alba* Leaf in Response to Herbivore and Mechanical Injuries

José L. S. de Almeida Clécio S. Ramos*

Department of Chemistry, Rural Federal University of Pernambuco, 52.171-030, Recife-Pe, Brazil

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ABSTRACT

There is no doubt that the chemical composition of plants, including nor-volatile and volatile compounds, is widely affected by abiotic and biotic stress. Plants are able to biosynthesize a variety of secondary metabolites against actions of natural enemies, such as herbivores, fungus, virus and bacteria. The present study revealed that the chemical compositions of leaf essential oils from *Ipomoea alba* underwent quantitative and qualitative alterations both when infested with the grasshopper *Elaeochlora trilineata* and mechanically damaged. Grasshopper attack and mechanical wounding induced the biosynthesis of nine volatile compounds in leaves of *I. alba*: cumene, α-ylangene, β-panasinsene, β-gurjunene aromadendrene, β-funebrene, spirolepechinene, cubenol and sclareolide. The amount of germacrene D (33.2% to 20.4%) decreased when the leaves were mechanically damaged; but when the leaves were attacked by a grasshopper, the germacrene D increased from 33.2% to 39.4%. The results showed that *I. alba* leaves clearly responded to abiotic and biotic stress and contribute to an understanding of plant responses to stress conditions.

1. Introduction

The chemical composition of plants can vary greatly in response to abiotic and biotic stress, such as soil salinity, temperature, humidity and nutrient deficiency, pathogen infection and herbivore attack [1,2]. In particular, plants biosynthesize a wide spectrum of secondary metabolites when they are injured by herbivores, including volatile compounds that consist predominantly of terpenes and widely known as allelochemicals [3,4].

Terpenes represent the main and most abundant class of plant secondary compounds, with approximately 55,000 elucidated chemical structures. Volatile terpenes are associated with abiotic and biotic stress tolerance [5,6]. Monoterpene linalool and sesquiterpene (E)-β-farnesene, biosynthesized by plants, act as allomones in herbivores and aphids, respectively [7]. Terpenes (-)-α-bisabolol, carvacrol, (+)-terpinen-4-ol and thymol are active against the Colorado potato beetle (*Leptinotarsa decemlineata*), whereas terpenes (-)-verbenone, camphor and linalyl

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*Corresponding Author:
Clécio S. Ramos,
Department of Chemistry, Rural Federal University of Pernambuco, 52.171-030, Recife-Pe, Brazil;
Email: clecio.ramos@ufrpe.com

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acetate have shown antifeedant activities [8]. *Chelonus insularis*, a wasp egg-larval endoparasitoid, is attracted to a blend of terpenes *α*-pinene, α-longipinene and *α*-copaene emitted by maize plants, mainly when the plant is damaged by *Spodoptera frugiperda* larvae [9].

Our previous studies showed that other classes of secondary metabolites, such as phenylpropanoids, have been related to the induced response of plants to biotic and abiotic stress. For example; Phenylpropanoids myristicin, dilapiol, eugenol and eugenol acetate were only identified in the essential oil of *Mangifera indica* leaves damaged by the grasshopper *Tropidacris Collaris* and mechanically [10]. Phenylpropanoid dillapiole has been previously reported in the essential oil of the *Solanum paniculatum* leaf as the plant's chemical defense to abiotic stress [11]. Biotic and abiotic stress can also affect the biological potential of plants as in the case of *Piper marginatum* leaves: *P. marginatum* essential oil obtained from the leaves damaged by insects showed lower antimicrobial potential against fungi and bacteria when compared to essential oil obtained from healthy leaves [12].

In our ongoing study to identify compounds associated with plant chemical defense [13], here we describe changes in the chemical profile of essential oils obtained from *Ipomoea alba* L. leaves after a grasshopper (*Elaeochlora trilineata*) attack or by mechanical injury. *I. alba*, popularly known as the moonflower or moonvine, is native to the Caribbean, Mexico and Brazil, where the plant is used in folk medicine due to its purgative, analgesic, anti-inflammatory, anti-rheumatism, antimicrobial, anti-pyretic, hypotensive and emollient properties [14,15].

There are few reports on chemical studies of *I. alba.* Indolizine alkaloids and glycoside have previously been isolated from *I. alba* seeds [16,17]. In relation to studies on the chemistry of the essential oils from the *I. alba* leaf on the biotic and abiotic stress, to our knowledge there are no reports.

2. Material and Method

2.1 Plant and Insect

Fresh leaves from *I. alba* were collected on Campus of Federal Rural University of Pernambuco (UFRPE) to obtain their essential oils. The plant was identified by Dr. Margareth F. de Sales of the same university and a voucher of species was deposited at the Vasconcelos Sobrinho Herbarium of the Federal Rural University of Pernambuco, Recife city, Brazil. Adult grasshoppers of the *species Elaeochlora trilineata* were collected on the campus of UFRPE and identified by Dr Argus Vasconcelos de Almeida (Department of Biology, UFRPE).

2.2 Stress Conditions

Adult *I. alba* stems with undamaged and insect-free leaves were collected from specimens (N = 3) growing on the campus of the Federal Rural University of Pernambuco (Figure 1) and maintained for 24 h in bottles with water in a greenhouse without supplementary lighting. Collected leaves (3 bottles) were infested during 4 hours with the *E. trilineata* grasshopper (10 specimens to each sample) to simulate biotic stress. Mechanical damage was caused by piercing leaf tissues (3 bottles) with a paper punch to simulate abiotic stress over a period of 4 h. Five 80 mm holes were punched in each leaf. Plant samples (N = 3) with healthy and undamaged leaves were collected and used as negative control.

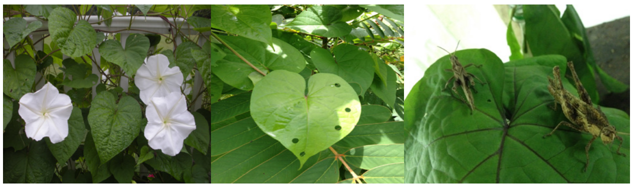

Figure 1. *I. alba* leaves healthy and submitted to abiotic and biotic stress.

2.3 Essential Oil Extraction

The essential oils from the undamaged leaves of the *I. alba* as well as those under biotic and abiotic stress (400 g each), were extracted using a modified Clevenger-type apparatus and subject to hydrodistillation for 2 h. The oil layers were separated and dried over anhydrous sodium sulfate, stored in hermetically sealed glass containers, and kept under refrigeration at 5 ºC until analysis. All experiments were carried out in triplicate.

2.4 Quantitative Analysis of Essential Oils

Analysis by gas chromatography (GC) was performed in a Hewlett Packard 5890 Series II GC machine equipped with a flame ionization detector (FID) and a non-polar DB-5 fused silica capillary column (30 m × 0.25 mm × 0.25 µm film thickness). The oven temperature was programmed to rise from 50 °C to 250 °C at a rate 3 C/min for integration purposes. Injector and detector temperatures were 250 °C. H_2 was used as the carrier gas at a flow rate of 1 L/min and 30 psi. inlet pressure in split mode (1:20). The injection volume was 1.0 µL of dilution solution (1/100) of oil in hexane. The relative concentrations of the identified constituents were calculated by base of the GC peak areas in the order of DB-5 column elution and expressed as a relative percentage of the total area of all the peaks. All the oil samples were analyzed in triplicate.

2.5 Chemical Identification of Essential Oils

The essential oils were analysed by gas chromatography/mass spectrometry (GC/MS) (50-250 ºC at 3 ºC min. rate) using a Hewlett-Packard GC/MS system (CG:5890 Series II/CG–MS: MSD 5971) equipped with a fused-silica capillary column (30 m × 0.25 mm i.d. × 0.25 µm) coated with DB-5column. The injector and detector temperatures were 250 °C and 260 °C, respectively. MS spectra were obtained using electron impact at 70 eV with a scan interval of 0.5 s, fragments from 40 Da to 550 Da, He carrier gas, flow rate 1 mL/min, split mode (1:20), injection volume 1 µL of dilution solution (1/100) of oil in hexane. Compound identification was established in retention indices with reference to a homologous series of C11–C24 n-alkanes, calculated using the Van den Dool and Kratz equation and by comparison with the GC/MS library mass spectra acquired from the data system [18,19].

3. Results and Discussion

The chromatograms obtained by GC/MS of essential oil samples from healthy and damaged leaves of *I. alba* showed qualitative and quantitative differences when compared to each other (Figure 2). Previous studies have revealed that the chemical composition of essential oils from *Ipomoea* species is constituted mainly of sesquiterpenes such as germacrene, cis-cadin-1(6),4-diene, humulene, caryophyllene, copaene, bicyclogermacrene, cadinol and murolene [20]. To our knowledge, there has been only one study on the chemistry of the essential oil from *I. alba* leaves, where the major constituent was identified as germacrene D (leaves 27.8% and flowers 45.8%) [20].

It was possible to identify sixteen compounds: four terpenes: δ-elemene, *E*-caryophyllene, germacrene D and c*is*-cadina-1,4-diene were found in the three oils. Sesquiterpene germacrene D was identified as the major compound with 39.4%, for leaves under biotic stress; 20.4%, for leaves under abiotic stress, and 33.2%, for healthy leaves (Table 1). Terpenes represent the largest and structurally the most diverse group of volatiles released by plants and are emitted either constitutively or induced in response to abiotic and biotic stresses [21]. Germacrene D commonly occurs in plants and has been identified in angiosperms, gymnosperms and bryophytes. Germacrene D is a precursor of various sesquiterpenes and it has been reported to have repellent and insecticidal activity [22]. For example, germacrene D has been

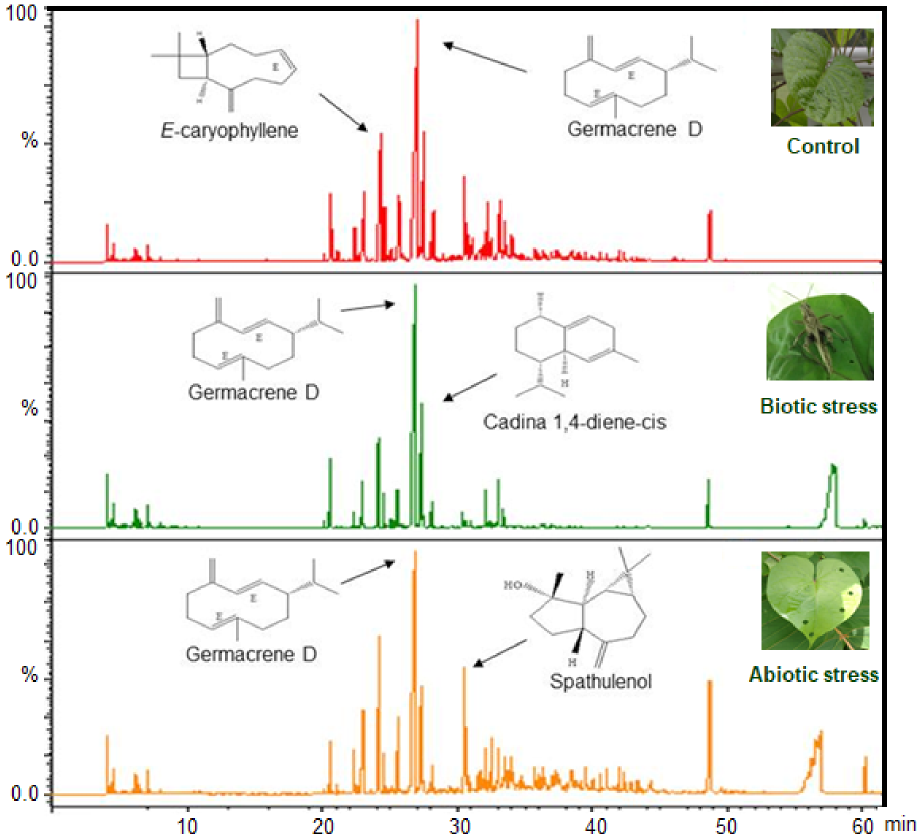

Figure 2. Chromatograms obtained by GC/MS of essential oils from *I. alba* leaves: healthy (Control) and damaged (Biotic and abiotic stress)

reported to have mosquitocidal activity against three mosquito species, *Anopheles gambiae*, *Culex quinquefasciatus* and *Aedes aegypti* [23].

Here, a total of nine volatile organic compounds have been identified in response to biotic and abiotic stress: cumene, *α*-ylangene, *β*-panasinsene, *β*-gurjunene aromadendrene, *β*-funebrene, spirolepechinene, cubenol and sclareolide.

In the essential oil from the biotic-stressed, herbivore-damaged leaf, the presence of only two compounds has been identified that had not been found in the essential oil samples from undamaged or mechanically-damaged leaves: *β*-Gurjunene (2.1%) and spirolepechinene (2.5%), associated with the inducible defense of *I. alba* in response to damage by the grasshopper *E. trilineata*. Essential oil extracted from the *Dipsacus asper* roots exhibited larvicidal activity against *Aedes aegypti* and *Culex pipiens; β*-Gurjunene has been identified as one of the major compounds in the oil [24]. The spirolepechinene is a spirosesquiterpene found in *Lepechinia bullata* and of a unique structure [25].

The *β*-funebrene and sclareolide were produced by the leaves of *I. alba* in significant quantities in response to injuries by the grasshopper and by mechanical injury. Sclareolide is a sesquiterpene lactone with antimicrobial and cytotoxic activity. It is derived from various plant sources including *Salvia sclarea* and *Nicotiana tabacum* [26]. *β*-funebrene has been identified as one of the main compounds in the essential oil of *Campomanesia adamantium* leaves, a plant used for its antiinflammatory, anti-diarrhoeal and antimicrobial activities [27].

A great number of compounds have been identified for essential oil from *I. alba* leaves in response to abiotic stress (cumene, *α*-ylangene, *β*-panasinsene, *β*-funebrene, aromadendrene, cubenol and sclareolide). Sesquiterpene *α*-ylangene has been identified in some essential oils of plants with diverse biological activities, but only as a minor compound [28]. Essential oil of *Eucalyptus globulus* has been reported to have insecticidal activity against insecticidal on *Acanthoscelides obtectus* adults as well as against the red mite *Dermanyssus gallinae;* some terpenes, including aromadendrene, have been associated with

Table 1. Chemical constituents of essential oils from healthy (Control) and damaged (Abiotic and biotic stress) *I. alba* leaves

Compounds	**RI^a**	**RI^b**	**Essential oils (%)^c**		
			Control	**Abiotic**	**Biotic**
1.Cumene	993	924		0.7± 0.02	1.1 ± 0.01
2.*δ*-Elemene	1331	1335	4.2 ± 0.01	0.7 ± 0.00	4.7± 0.01
3.α-Ylangene	1372	1373		1.7 ± 0.02	
4.*β*-Panasinsene	1385	1382		1.1 ± 0.03	
5.*β*-Elemene	1389	1389	3.3 ± 0.04	4.0 ± 0.01	
6.*β*-Funebrene	1418	1413		6.3 ± 0.02	6.1 ± 0.02
7.*E*-Caryophyllene	1420	1417	13.6 ± 0.01	5.2 ± 0.02	3.9 ± 0.03
8.*β*-Gurjunene	1428	1431			2.1± 0.04
9.Spirolepechinene	1454	1449	3.7 ± 0.03		
10.Aromadendrene	1453	1458		3.5 ± 0.03	
11.Spirolepechinene	1451	1449			2.5
12.Germacrene D	1482	1484	33.2 ± 0.00	20.4 ± 0.01	39.4 ± 0.01
13.*Cis*-cadina-1,4-diene	1497	1495	10.3 ± 0.02	5.2 ± 0.02	11.8 ± 0.01
14.Spathulenol	1580	1577	4.1 ± 0.02	9.8 ± 0.01	
15.Cubenol	1629	1627		2.3 ± 0.02	
16.Sclareolide	2108	2065		6.7 ± 0.01	3.3 ± 0.02
	Total (%)		72.4 ± 0.01	67.5 ± 0.02	74.8 ± 0.02
	Oil yields (%)		0.19 ± 0.02	0.23 ± 0.01	0.20 ± 0.01

^{a}Retention indices calculated from retention times in relation to those of the series n-alkanes on a 30 m DB-5 capillary column. ^{b}Retention indices from the literature ^{c}Composition: total area percentages given as mean ± SD (*n* = 3).

oil activity [29]. The essential oil of the *Blepharocalyx cruckshanksii* leaves with 24.5% of cubenol showed insecticidal, repellent and anti-food activity against the horn fly, *Haematobia irritans*, considered the largest livestock pest in the world [30].

In our study, the main quantitative variations between the essential oils of damaged and healthy leaves consisted among their major compounds. In the healthy leaves, the principal component was identified as germacrene D. This decreased when the leaves suffered mechanical damage. A significant increase in the amount of germacrene D (33.2% to 39.4%) was observed when the leaves were injured by the grasshopper *E. trilineata*.

4. Conclusions

In summary, we identified nine volatiles compounds from essential oils of *I. alba* leaves in response to abiotic and biotic stress. The results obtained contribute significantly to knowledge about plant chemistry under stress conditions, mainly for the genus *Ipomoea*.

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Conflict of Interest

No potential conflict of interest was reported by the authors.

References

[1] Morales-Tapia, P., Cabrera-Barjas, G., Giordano, A., 2021. Polyphenolic distribution in organs of *Argylia radiata*, an extremophile plant from Chilean Atacama desert. Natural Product Research. 35, 4143-4147.

[2] Fichman, Y., Mittler, R., 2020. Rapid systemic signaling during abiotic and biotic stresses: is the ROS wave master of all trades? Plant Journal. 102(5), 887-896.

[3] De Lange, E.S., Laplanche, D., Guo, H., et al., 2020. *Spodoptera frugiperda* Caterpillars Suppress Herbivore-Induced volatiles Emissions in Maize. Journal of Chemical Ecology. 46(3), 344-360.

[4] Ulhoa, L.A., Barrigossi, J.A.F., Borges, M., et al., 2020. Differential induction of volatiles in rice plants by two stink bug species influence behaviour of conspecifics and their natural enemy *Telenomus podisi*. Entomologia Experimentalis et Applicata. 168(1), 76-90.

[5] Boncan, D.A.T., Tsang, S.S., Li, C., et al., 2020. Terpenes and terpenoids in plants: Interactions with environment and insects. International Journal of Molecular Sciences. 21(19), 7382.

[6] Schwab, W., Fuchs, C., Huang, F.C., 2013. Transformation of terpenes into fine chemicals. European journal of lipid science and technology. 115(1), 3-8.

[7] Gonzalo, E.C., Raúl, S.V., Isabel, B.M., et al., 2017. Antifeedant effects of common terpenes from Mediterranean aromatic plants on Leptinotarsa decemlineata. Journal of soil science and plant nutrition. 17(2), 475-485.

[8] Sharma, E., Anand, G., Kapoor, R., 2017. Terpenoids in plant and arbuscular mycorrhiza-reinforced defence against herbivorous insects. Annals of Botany. 119(5), 791-801.

[9] Fabian, R., Rojas, J.C., Cisneros, J., et al., 2019. Herbivore-Induced Volatiles from Maize Plants Attract *Chelonus insularis*, an Egg-Larval Parasitoid of the Fall Armyworm Ortiz-Carreon. Journal of Chemical Ecology. 45(3), 326-337.

[10] Silva, R.R., Câmara, C.A.G., Almeida, A.V., et al., 2012. Biotic and abiotic stress-induced phenylpropanoids in leaves of the mango (*Mangifera indica* L., Anacardiaceae). Journal of the Brazilian Chemical Society. 23(2), 206-2011.

[11] Ramos, N.S.M., Ramos, C.S., 2013. Volatiles from *Solanum paniculatum* Leaves in Response to Mechanical Damage. Chemistry of Natural Compounds. 49, 953-954.

[12] Silva, A.S., Silva, M.A., Almeida, A.V., et al., 2016. Herbivory causes chemical and biological changes on essential oil from *Piper marginatum* leaves. The Natural Products Journal. 6, 1-5.

[13] Silva, R.R., Moraes, M.M., Câmara, C.A.G., et al., 2015. Change in the profile chemical of leaves *Mangifera indica* after their metabolism on grasshopper *Tropidacris collaris*. Natural Product Communications. 10, 1809-1810.

[14] Lawson, S.K., Davis, M.N., Brazell, C., et al., 2017. Chloroform extracts of *Ipomoea alba* and *Ipomoea tricolor* Seeds Show Strong in-vitro antibacterial, antifungal, and cytotoxic activity. Journal of Pharmacognosy and Phytochemistry. 6(5), 730-734.

[15] Brasileiro, B.G., Pizziolo, V.R., Raslan, D.S., 2006. Antimicrobial and cytotoxic activities screening of some Brazilian medicinal plants used in Governador Valadares district. Brazilian Journal of Pharmaceutical Sciences. 42, 195-202.

[16] Castaneda-Gomez, J., Rosas-Ramirez, D., Cruz-Morales, S., et al., 2017. HPLC-MS profiling of the multidrug-resistance modifying resin glycoside content of *Ipomoea alba* seeds. Revista Brasileira de Farmacognosia. 27(4), 434-439.

[17] Ikhiri, K., Koulodo, D.D.D., Garba, M., et al., 1987. New indolizine alkaloids from *Ipomoea alba*. Journal of Natural Products. 50(2), 152-156.

[18] Adams, R.P., 2007. Identification of essential oil components by gas chromatography/ quadrupole mass spectroscopy. Allured Publishing Corporation. Carol Stream. Illinois. pp. 468.

[19] Van Den Dool, H., Kratz, P.D., 1963. A Generalization of the Retention Index System Including Linear Temperature Programmed Gas-Liquid Partition Chromatography. Journal of Chromatography A. 11, 463-471.

[20] Tenório, T.M., Moraes, M.M., Camara, C.A., et al., 2021. Scents from the Brazilian Atlantic Forest Biome: chemical composition of essential oils from the leaves and flowers of seven species of *Ipomoea* (Convolvulaceae). Journal of Essential Oil Research. 33(6), 567-583.

[21] Esha Sharma, G.A., Rupam, K., 2017. Terpenoids in plant and arbuscular mycorrhiza-reinforced defence against herbivorous insects. Annals of Botany. 119, 791-801.

[22] Noge, K., Becerra, J.X., 2009. Germacrene D, a common sesquiterpene in the genus *Burser*a (Burseraceae). Molecules. 14(12), 5289-5297.

[23] Ravi Kiran, S., Sita Devi, P., 2007. Evaluation of mosquitocidal activity of essential oil and sesquiterpenes from leaves of *Chloroxylon swietenia* DC. Parasitology research. 101(2), 413-418.

[24] Lu, H.Y., Liu, X.C., Liu, Q.Z., et al., 2017. Chemical composition of *Dipsacus asper* Wallich ex Candolle (Dipsacaceae) essential oil and its activity against mosquito larvae of *Aedes aegypti* and *Culex pipiens* pallens. Tropical Journal of Pharmaceutical Research. 16(1), 179-184.

[25] Chappell, J., O'Maille, P.E., Noel, J.P., 2006. Biosynthetic potential of sesquiterpene synthases: alternative products of tobacco 5-epi-aristolochene synthase. Archives of Biochemistry and Biophysics. 448, 73-82.

[26] Hayet, E., Fatma, B., Souhir, I., et al., 2007. Antibacterial and cytotoxic activity of the acetone extract of the flowers of *Salvia sclarea* and some natural products. Pakistan Journal Pharmaceutical Science. 20, 146-148.

[27] Sá, S., Chaul, L.T., Alves, V.F., et al., 2018. Phytochemistry and antimicrobial activity of Campomanesia adamantium. Revista Brasileira de Farmacognosia. 28, 303-311.

[28] Basile, S., Badalamenti, N., Riccobono, O., et al., 2022. Chemical Composition and Evaluation of Insecticidal Activity of *Calendula incana* subsp. *maritima* and *Laserpitium siler* subsp. *siculum* Essential Oils against Stored Products Pests. Molecules. 27(3), 588.

[29] Hikal, W.M., Baeshen, R.S., Said-Al Ahl, H.A., 2017. Botanical insecticide as simple extractives for pest control. Cogent Biology. 3, 1404274.

[30] Espinoza, J., Medina, C., Aniñir, W., et al., 2021. Insecticidal, Repellent and Antifeedant Activity of Essential Oils from *Blepharocalyx cruckshanksii* (Hook. & Arn.) Nied. Leaves and *Pilgerodendron uviferum* (D. Don) Florin Heartwood against Horn Flies, *Haematobia irritans* (Diptera: Muscidae). Molecules. 26(22), 6936.