Essential oil of *Baccharis* as a sustainable alternative for small farmers in South America

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Abstract— *This work aimed at evaluating the insecticidal and repellent effect of essential oils of Baccharis trimera and Baccharis articulate leaves against the maize weevil in maize grains. The essential oil was extracted by hydrodistillation and the chemical composition showed that the major constituent was the carquejil acetate (73.6%) for B. trimera and β-pinene (22.3%) and β-cariofilene (21.5%) for B. articulate. Our results show that B. trimera oil was insecticidal and repellent to maize weevil. About 100% of mortality was achieved using concentrations of 0.32 µL.cm-2, after 24 h of exposure and the lower concentration 0.065 µL.cm-2 obtained similar results in 96 h. The LD50 value for B. trimera essential oil was 8.4 µL/ Petri dishes or 0.05 µL.cm-2. The results obtained indicate that the effect insecticidal of the essential oil of B. trimera is probably because of the presence of carquejil acetate. B. articulate showed low insecticidal activity. The values of the Preference level varied from -0.6 (0.065 µL.cm-2) to -0.9 (0.65 µL.cm-2) for B. trimera oil and -0.4 (0.065 µL.cm-2) to -0.7 (0.65 µL.cm-2) for B. articulata oil, being lower than -0.10. The minimum limit to consider that a plant has repellency activity, demonstrating that the both plants oils presents repellency activity. Although from an economical point of view synthetic chemicals are still more frequently used as repellents, we find the essential oil of B. trimera have potential to be used sustainably as bioinseticide by the small farmers.*

***Keywords*— Baccharis trimera, Baccharis articulata, repellency activity, insecticidal activity, small farmer, sustainability.**

I. INTRODUCTION

In the world, pests (especially weeds, pathogens and insects) are the highest competitor of agricultural crops, reducing drastically the production in the range of 25–50% [1, 2]. Among the most important pests, weeds alone are responsible for nearly 34% of reduction in crop yields. Aiming at protecting the agricultural crops, high amounts of synthetic pesticides are used around the world. Nowadays, the maize weevil is the most relevant pest found during maize grains storage, especially in the small properties, where storage conditions are not adequated [2].

Aromatic plants and their essential oils have been used since antiquity in flavor and fragrances, as condiment or spice, in medicines, as antimicrobial/insecticidal agents, and to repel insect or protect stored products [3]. These oils represent effective alternatives to synthetic pesticides without producing adverse effects on the environment [4]. The essential oils of plants have been broadly studied for pest-control including toxic and repellent effects, antifeedant, ovicidal and other properties [5, 6].

The species of the genus Baccharis, belonging to the Asteraceae family, being from South America, probably the Peruvian Andes. *Baccharis trimera* (Less) DC or "bitter broom" as it is popularly known, is a native shrub of the fields and forests of the southern borders of Brazil, Bolivia, Paraguay, Uruguay and northern Argentina [7]. It has pharmacological actions such as anti-inflammatory and antioxidant [8, 9]. *Baccharis articulata* (Lam.) Pers. is a shrub much like *Baccharis trimera*, originally from South America, taking place in Brazil, Paraguay, Uruguay and northern and central Argentina [7]. The essential oil has the L-limonene constituents, alpha-gurjuneno, trans-caryophyllene, Germacrene, bicyclogermacrene, spathulenol, beta pinene, globulol, viridiflorol, among others, being the major compound Beta-pinene [10].

In this context, the aim of this study was to evaluate the bioactivity and repellency effect of essential oil of *Baccharis trimera* and *Baccharis articulata* against the *Sitophilus zeamais* in maize grains.

II. MATERIAL AND METHOD

2.1 Plant Materials

Leaves of *Baccharis trimera* and *Baccharis articulata* were collected in summer in Erechim (RS, south of Brazil). The identity of the plants was confirmed by the Balduino Rambo Herbarium of the URI in Erechim, south of Brazil (n° HPBR: 11.501 - *B. articulata* and HPBR: 11.470 - *B. trimera*).

2.2 Procedure for Isolation and Characterization of the Essential Oils

The plant material was dried naturally on laboratory benches at room temperature (30°C) for 5 d until crisp. The dried material was stored at room temperature and then hydro-distilled to extract the essential oil using a Clevenger-type apparatus for 2h. The extraction conditions were established as 100g of air-dried sample and 1:30 plant material/water volume ratio. Anhydrous sodium sulphate was used to remove the water after extraction. The extracted oil was stored in freezer at -20°C.

The chromatographic analyses of the essential oils were performed using a Shimadzu - QP 5050A series gas chromatograph (GC-MS), using a DB-5 fused silica capillary column (30m x 0.25mm i.d. x 0.25µm film thickness). The temperature program used for the analysis was: initial temperature at 60°C, held for 3min, and ramped at 3°C.min^{-1} to 300 °C. Helium was used as carrier gas at a flow rate of 1mL.min^{-1}. The detector temperature was set to 300°C, and the injector temperature 240°C with injection port (split 1:20). The oil compounds were identified by comparison of mass spectra of each peak with those of authentic samples in a mass spectra library (The Wiley Registry of Mass Spectral Data, 7th ed.).

2.3 Insects

Sitophilus zeamais was cultured in a controlled temperature (25±1°C) and at 70–75% of relative air humidity (rh) in darkness. The food media used were whole maize grains. The adults used in the experiment were 2–3 weeks post-emergence.

2.4 Contact Toxicity on Filter Paper and Lethal Doses Assessment

The contact effect of essential oils against maize weevil was evaluated on filter paper discs (Whatman N°1) (14 cm diameter, surface 153.9 cm^2), treated with different dosages of oil. The filter papers were placed in glass Petri dishes (14 cm diameter, 1.5 cm height and volume 0.23 L). An aliquot of 0, 10µL, 20µL, 30µL, 50µL and 100µL of oil was applied to the filter paper discs corresponding to dosages of 0, 0,005, 0,13, 0,19, 0,32 and 0,65 µL.cm^{-2}. An amount of 50 unsexed adults maize weevils was used separately into each dish and these were kept in darkness in the laboratory at 25±1°C and 70–75% rh [11].

Each treatment was replicated at least six times. Insect mortalities were analyzed after 24 h. The percentage of insect mortality was calculated using the Abbott correction formula for natural mortality in untreated controls [12]. The different 50% lethal doses (LD50) were calculated using linear regression analysis, at 24h, based on the supracited experimental data; contact Toxicity on Filter Paper [13].

After that, the effect of the insect mortality against time (0, 4, 8, 12h, 24, 48, 72, 92 and 120 h) with an aliquot of 0, 10 µL, 20 µL, 30 µL, 50 µL and 100 µL (0, 0,005, 0,13, 0,19, 0,32 and 0,65 µL.cm^{-2}), was studied. The treatment was replicated six times in similar conditions used in the Contact Toxicity study on filter paper experiment.

2.5 Repellency Bioassay

The values of effect of essential oils against maize weevil were evaluated six times in the repellence bioassay. The repellence action of the essential oils was carried out using a system containing five Petri plates (diameter=14 cm, 153.9 cm^2), codified as A, B, C, D and E. In plates A and B, 20 g of maize with different aliquot (10, 20, 30, 50 and 100µL), corresponding to dosages of 0.065, 0.13, 0.19, 0.32 e 0.65 µL.cm^{-2} of the essential oils, were added. In plates C and D (blanks) only the substrate was added and in plate codified as E (central), 20 adults of maize weevil were delivered and after 24 h the number of insects were counted. Each dish was kept in darkness at 25±1oC and 70–75% of rh. Aiming at comparing the different treatments, the Preference level (PL) [11] was defined as $_{(1)}$:

$$PL = \frac{\%of\ insects\ of\ test\ plants - \%of\ insects\ of\ blank\ experiment}{\%of\ insects\ of\ test\ plants + \%of\ insects\ of\ blank\ experiment} \tag{1}$$

Where:

PL of -1.00 to -0.10, test plant repellent; PL of -0.10 to +0.10, test plant neutral; PL of+0.10 to +1.00, test plant attractant.

2.6 Repellency Bioassay simulating small bins

The values of effect of essential oils against maize weevil were evaluated five times in the repellence bioassay (similar to previous). However, in this case, the repellence action of the essential oils was carried out using a system containing five small glass bins (diameter=4.5 cm, height= 6 cm and total area=95,4 cm^3), codified as A, B, C, D and E. In plates A and B, 50 g of maize with different aliquot (50, 100, 150, 250, 400 e 500 $\mu L.bins^{-1}$), corresponding to dosages of 0.52, 1.05, 1.57, 2.62, 4.19 and 5.24 $\mu L.cm^{-3}$ of the essential oils, were added. In plates C and D (blanks) only the substrate was added and in plate codified as E (central), 50 adults of maize weevil were delivered and after 24 h the number of insects were counted. The treatment was replicated five times in similar conditions of that used in the Contact Toxicity on filter paper experiment. Each small glass bins was kept in darkness at 25±1^{o}C and 70–75% of rh. Aiming at comparing the different treatments, the Preference level (PL) was established, as cited by Procópio [11].

2.7 Statistical Analysis

Data were analyzed by analysis of variance (ANOVA). Mean separation was determined by the Tukey's test as a confidence level of 95%. Pearson correlations were also used.

III. RESULTS AND DISCUSSION

3.1 Chemical Constituents of the Essential Oils

The yield of the essential oils obtained by hydro-distillation was: 1.5% (v/w) for *Baccharis trimera* and 0.6% (v/w) for *Baccharis articulata*. Table 1 show the essencial oil of *B. trimera* presented 75.5% of monoterpenes oxygenated, 12.1% of Oxygenated Sesquiterpenes, 7.3% Monotrerpenes Hydrocarbons and the major constituent was the carquejil acetate (73.6%). The essencial oil of *B. articulata* presented 64% of Sesquiterpenes Hydrocarbons, 22.3% of Monotrerpenes Hydrocarbons, 9.8% of Oxygenated Sesquiterpenes and que major constituents were the β-pinene (22.3%) and β-cariofilene (21.5%). The results obtained in this work are similar to that presented in the current literature [14].

TABLE 1
RELATIVE PERCENTAGE TO THE COMPONENTS OF THE ESSENTIAL OIL OF BACCHARIS TRIMERA AND BACCHARIS ARTICULATE LEAVES.

Compound	*B. trimera*	*B. articulata*
1. β-pinene	4.65	22.28
2. limonene	2.03	-
3. *trans*-ocimene	0.63	-
4. Carquejil acetate	73.6	-
5. sabinil acetate	0.92	-
6. neril acetatoe	0.98	-
7. neoisolongifolene	-	7.60
8. β –elemene	0.15	-
9. guaia-3,9-diene	-	10.52
10. β-cariofilene	0.28	21.53
11. α-guaiene	-	
12. α-humulene	0.07	
13. aromadendrene	0.32	-
14. δ-gurjunene	0.06	-
15. germacrene-D	0.91	6.84
16. biciclo-germacrene	0.15	17.58
17. α-germacrene	0.04	
18. δ-cadinene	0.26	
19. elemol	1.16	-
20. espatulenol	0.90	9.85
21. viridiflorol	1.57	-
22. β-eudesmol	1.65	-
23. α –cadinol	1.39	-
24. palustrol	5.48	
Monotrerpenes Hydrocarbons	7.31	22.3
Monoterpenes oxygenated	75.5	-
Sesquiterpenes Hydrocarbons	2.2	64.1
Oxygenated Sesquiterpenes	12.15	9.8
Total	97.2	96.2

3.2 Filter Paper Contact Toxicity

Table 2 shows the results in terms of the efficiency of the treatments of insecticidal activity with different doses of essential oil of *Baccharis trimera* and *Baccharis articulata* against maize weevil in stored maize grains. One can observe that the essential oils of the *Baccharis trimera* presented activity, of about 88% of mortality in concentration of 0.13 $\mu L.cm^{-2}$ and 100% of mortality in concentration of 0.32 $\mu L.cm^{-2}$, after 24 h of exposure. *Baccharis articulate* showed low activity against *Sitophilus zeamais*.

The LD50 values for *B. trimera* essential oil against the maize weevil was 8.4 µL/ Petri dishes or 0.05 $\mu L.cm^{-2}$. In the probit analyses, the calculated regression line equation [13] for maize weevil was $y = 33.18Ln(x) – 20.46$ ($R^2 = 0.93$).

TABLE 2
EFFICIENCY OF THE TREATMENTS OF INSECTICIDAL ACTIVITY WITH DIFFERENT DOSES OF ESSENTIAL OIL OF DE BACCHARIS TRIMERA AND BACCHARIS ARTICULATA AGAINST MAIZE WEEVIL IN STORED MAIZE GRAINS IN 24 H.

Concentration ($\mu L.cm^{-2}$) and (µL/ Petri dishes)	Mortality (%)*	
	B. Trimera	*B. Articulata*
0 $\mu L.cm^{-2}$ (0µL/ Petri dishes)	$0^{f} \pm 0$	$0^{c} \pm 0$
0.013 $\mu L.cm^{-2}$ (2µL/ Petri dishes)	$2.2^{e} \pm 0.5$	$0^{c} \pm 0$
0.032 $\mu L.cm^{-2}$ (5µL/ Petri dishes)	$20.8^{d} \pm 1.5$	$0^{c} \pm 0$
0.065 $\mu L.cm^{-2}$ (10µL/ Petri dishes)	$69^{c} \pm 0.7$	$0^{c} \pm 0$
0.13 $\mu L.cm^{-2}$ (20µL/ Petri dishes)	$87.7^{b} \pm 2.5$	$0.7^{b} \pm 1.6$
0.19 $\mu L.cm^{-2}$ (30µL/ Petri dishes)	$93.8^{b} \pm 2$	$0.7^{b} \pm 1$
0.32 $\mu L.cm^{-2}$ (50µL/ Petri dishes)	$100^{a} \pm 0$	$0.7^{b} \pm 0.8$
0.65 $\mu L.cm^{-2}$ (100µL/ Petri dishes)	$100^{a} \pm 0$	$4.3^{a} \pm 0.5$

Means (standard deviations) in the same lines without a common letter are significantly different at 95% of confidence level (Tukey Test) and N=6

The study of effect of insect mortality in the time (0 to 120 h) showed that the concentration 0.32 $\mu L.cm^{-2}$ obtained about 100% mortality in 24 h and the lower concentration 0,065 $\mu L.cm^{-2}$ obtained similar results in 96 h (Figure 1). Tapondjou et al. [15] showed that 1.56$\mu L.cm^{-2}$ of essential oil of *E. saligna* was necessary to obtain 100% of mortality of *S. zeamais* in the fourth day of exposure and 0.78$\mu L.cm^{-2}$ of the essential oil of *Cupressus sempervirens* to obtain 100% of mortality in the fifth day.

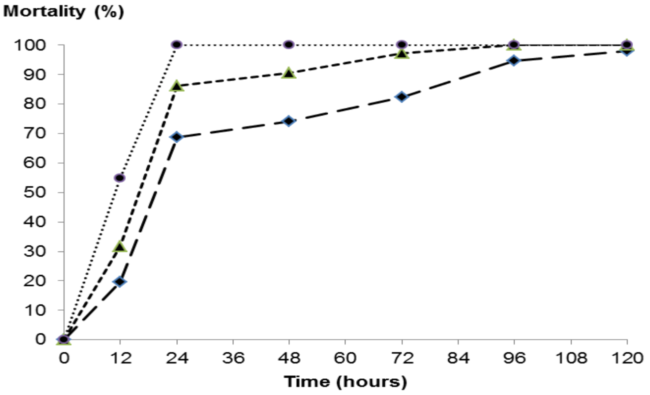

FIGURE 1. MORTALITY (%) OF MAIZE WEEVIL AT DIFFERENT CONCENTRATIONS OF BACCHARIS TRIMERA OIL AT 120 HOURS.

The essential oil of *B. trimera* showed high insecticidal activity an presented the major constituent carquejil acetate (73.6%) and the essential oil of *B. articulata* showed low insecticidal activity and presented the major constituent β-pinene (22.3%) and β-cariofilene (21.5%). The results obtained here are very interesting because the effect of the essential oil of *B. trimera* is probably because of the presence of carquejil acetate (73.6 %). The insecticidal activity of carquejil acetate (LD50) was similar or better the other compounds as safrole, 1,8-cineole and camphor.

3.3 Repellency activity

One can see, based on the preference level (PL) (Table 3), that the essential oils tested in this work, *B. trimera* and *B. articulata*, showed efficiency against the adults of maize weevil. For *B. articulata* oil is possible to notice a trend of increasing activity with increasing dosage and *B. trimera* oil showed activity at all doses tested.

TABLE 3
PREFERENCE LEVEL (PL) FOR THE ESSENTIAL OIL FROM B. TRIMERA AND B. ARTICULATA, FOLLOWING THE METHODOLOGY PROPOSED BY PROCÓPIO ET AL.[11].

Concentration (µL/ Petri dishes)/(µL.cm^{-2})	*B. trimera*		*B. articulata*	
	% of insect Treatment*	PL**	% of insect Treatment	PI
10/0.065	17.5^{a}±10.8	-0.6	31.8^{c}±12.7	-0.4
20/0.13	18.8^{a}±11.2	-0.6	35.0^{c}±8.3	-0.3
30/0.19	9.7^{a}±8.1	-0.8	19.3^{ab}±1.9	-0.6
50/0.32	9.8^{a}±8.3	-0.8	15.5^{a}±9.8	-0.7
100/0.65	6.1^{a}±7.6	-0.9	16.8^{a}±4.9	-0.7
Total	12.4^{a}±10.0		23.7^{b}±11.4	

**** Means (standard deviations) in the same lines without a common letter are significantly different at 95% of confidence level (Tukey Test) and N=6***

***** PI of -1.00 to -0.10, test plant repellent; PI of -0.10 to +0.10, test plant neutral; PI of+0.10 to +1.00, test plant attractant.***

The values of the PL varied from -0.6 (0.065 µL.cm^{-2}) to -0.9 (0.65 µL.cm^{-2}) for *B. trimera* oil and -0.4 (0.065 µL.cm^{-2}) to -0.7 (0.65 µL.cm^{-2}) for *B. articulata* oil, being lower than -0.10, the minimum limit proposed by Procópio et al. [11] to consider that a plant has repellency activity. Also was observed higher activity the essential oil of *B. trimera* (Table 3).

Procópio et al. [11] evaluated the repellency effect of fragments of leaves of six vegetables species against adults of maize weevil and verified, based on the PL, that two species caused repellency, the leaves of *Eucalyptus citriodora* (PI = -0.81) and *Capsicum frutescens* (Meliaceae) (PI= -0.17). Tested eucalyptus oils were highly repellent to the two insect species, *T. confusum* and *S. zeamais*.

The highly toxic and repellency effects of some of other main constituents of these oils such as camphor and safrole, terpineol and α-pinene have been demonstrated by other researchers [16, 17]. However there are no studies demonstrating insecticidal activity of carquejil acetate.

3.4 Repellency Bioassay simulating small bins

The Repellency Bioassay simulating small bins presented promising results at all aliquot tested oil, showing repellent effect that ranged of preference level from -0.8 (0.52 µL.cm^{-3}) to -1 (5.2 µL.cm^{-3}) for *B. trimera* oil and -0.7 (0.52 µL.cm^{-3}) to -0.9 (5.2 µL.cm^{-3}) for *B. articulata* oil (Table 4). The results suggest that the both essential oil could be used against maize weevil, however the *B. trimera* oil is most promising to use in large scale.

TABLE 4
PREFERENCE LEVEL (PL) FOR THE ESSENTIAL OIL FROM B. TRIMERA AND B. ARTICULATA IN SMALL BINS, FOLLOWING THE METHODOLOGY PROPOSED BY PROCÓPIO ET AL. [11].

Concentration	*B. trimera*	*B. articulata*
0.52 µL.cm^{-3} (50µL/ small bins)	-0.8*	-0.7
1.05 µL.cm^{-3} (100µL/ small bins)	-0.9	-0.8
1.57 µL.cm^{-3} (150µL/ small bins)	-0.9	-0.9
2.62 µL.cm^{-3} (250µL/ small bins)	-0.9	-0.9
5.24 µL.cm^{-3} (500 µL/ small bins)	-1	-0.9

**** PL of -1.00 to -0.10, test plant repellent; PL of -0.10 to +0.10, test plant neutral; PI of+0.10 to +1.00, test plant attractant.***

IV. CONCLUSION

The results permitted us to conclude that the essential oil of *B. trimera* presented high insecticidal and repellency against maize weevil. Although from an economical point of view synthetic chemicals are still more frequently used as repellents, natural products (essential oils) have the potential to provide efficient and safer repellents for humans and the environment. Large quantities of plant material would have to be processed for use in small-scale. In this case, the small farmers could cultivate their own biopesticides.

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