

Effects of Essential Oils from Two Species of Piperaceae on Parasitized and Unparasitized Eggs of *Oebalus insularis* (Heteroptera: Pentatomidae) by *Telenomus podisi* (Hymenoptera: Platygasteridae)

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Biological control of *Oebalus insularis* eggs is considered an important strategy in the control of this rice “stink bug”. Effects of essential oils (EOs) from *Piper jacquemontianum* Kunth and *Piper marginatum* Jacq. (Piperaceae) on the viability of parasitized and unparasitized eggs by *Telenomus podisi*, in concentrations of 0.5 to 2.0%, were evaluated. EOs from both species at a concentration of 2% affected the development of embryos of *O. insularis* as well as of *T. podisi* after 24 and 48h of their administration. In both species, *P. jacquemontianum* and *P. marginatum* LC₅₀, 24 h before the application of treatments on eggs of *O. insularis* was 3.835 y 3.037, respectively. LC₅₀ after 48 h of treatment with EOs showed contrasting results which varied from 2.207 and 1.811 for *P. jacquemontianum* and *P. marginatum*, respectively. LC₅₀ calculated before 24 h in parasitized eggs of *O. insularis* by *T. podisi*, was 3.037 and 2.171 for *P. jacquemontianum* y *P. marginatum*, respectively, while after 48h of treatment it was 1.166 y 1.935, respectively. Absorption of the EO components by the unparasitized and parasitized eggs of *O. insularis* by *T. podisi* was higher after 48 h of exposition. This is due to the longer exposure time, which allows higher penetration of the EOs into the interior of insect eggs by microscopic pores and micropyle.

Keywords: *Oebalus insularis*, Pentatomidae, *Telenomus podisi*, Platygasteridae, Piperaceae, *Piper jacquemontianum*, *Piper marginatum*.

The “Rice Stink Bug” *Oebalus insularis* Stal, is one of the most important pests in the rice crop in Panama and Central America. [1-2]. In addition, it is considered as an invasive pest in the state of Florida (USA), present in all rice fields since 2007 [1,3]. The damage is caused by the nymphs from second instar stage to adult phase by inoculating toxins and phytopathogens and by sucking the contents of grains, in milky phenological stage [4]. This process facilitates the entry of fungi of different genera [5], evoking a symptomatology called “stained grains”, which reduces significantly the production of this crop. The parasitism of *Telenomus podisi* Ashmead, in the egg phase of *O. insularis*, is the most efficient option for reducing their population, assuring the sustainability of rice crops [2,6]. Therefore, the utilization of EOs as natural insecticides obtained from species of *Piper* such as *Piper jacquemontianum* Kunth and *Piper marginatum* Jacq. (Table 1), may offer a valuable alternative for the management of *O. insularis*, thus reducing the use of synthetic insecticides in the cultivated areas of rice in Panama. Therefore, this study aims at evaluating the effects of EOs from these two species at concentrations from 0.25 to 2.0%, on the inviability of parasitized and unparasitized eggs of *O. insularis* by *T. podisi*.

Chemical composition of these EOs has been previously studied by our group and the Table 1 summarizes their principal components [7-9]. The results obtained indicate that at a concentration of 2% of EOs from *P. jacquemontianum* and *P. marginatum*, showed a higher rate of inviability of eggs than 50.0% (Table 2), in addition to confirming that the rate of inviability of eggs of *O. insularis* increases as the period of development of embryo increases to 48 hours (Table 2).

Table 1: Main components of essential oils from the leaves of *P. jacquemontianum* and *P. marginatum* [7-9].

Species	Compound	RI _{calc}	RI _{lit}	Concentration (%)	Voucher No
<i>P. jacquemontianum</i>	Linalool	1100 ¹	1100 ²	14.5	Florpan
	α -Phellandrene	1003	1003	13.8	6611
	Limonene	1031	1031	12.2	
	β -Pinene	961	974	10.1	
	α -Pinene	919	931	9.6	
	<i>p</i> -Cymene	1027	1025	7.4	
<i>P. marginatum</i>	6E-Nerolidol	1563	1571	4.6	
	Z-Isosafrol	1304	1298	34.4	Florpan
	Myristicine derivative (Croweeacin)	1458	1460	10.7	8367
	γ -Terpinene	1003	1015	10.5	

¹RI_{calc}=Calculated Retention Index. ²RI_{lit}=Literature retention Index [11]

Table 2: Rate of inviability unparasitized eggs of *Oebalus insularis* Stal, after the application of essential oils from two species of *Piper*.

Concentration (%)	<i>Piper jacquemontianum</i>		<i>Piper marginatum</i>	
	24 h.	48 h.	24 h.	48 h.
2.0	54±7.9 a ¹	69±8.2 a	64±4.3 a	89±2.2 a
1.0	31±3.5 b	40±6.5 b	32±3.6 b	59±5.5 b
0.5	17±3.7 c	19±3.8 c	20±2.6 c	48±3.9 c
0.25	14±3.5 c	17±4.2 c	8±1.3 d	23±4.2 d
Tween	0.00 d	0.00 d	0.00 e	0.00 e
H ₂ O	0.00 d	0.00 d	0.00 e	0.00 e
H	55.508	56.040	62.582	61.345
p-value	< 0.0001	< 0.0001	< 0.0001	< 0.0001
LC ₅₀	3.835	2.207	3.037	1.811
CI	3.61-4.05	2.15-2.25	2.78-3.28	1.74-1.87

¹Means indicated by the same lowercase superscripts, are different among themselves. LC₅₀=Lethal concentration (50%). CI = 95% Confidence Interval.

Previous studies have documented the ovicidal effect of EOs from some species of Piperaceae such as *Piper aduncum* L. on *Euchistus heros* (Fabricius) (Pentatomidae). The ovicidal activity is due to the presence of several compounds in Piperaceae EOs, especially dillapiole, myristicin, asaricine, sphathuonol and piperitone [10].

Table 3: Rate of inviable eggs of *Oebalus insularis*, parasitized by *Telenomus podisi*, after application of different concentrations of essential oils of Piperaceae.

Concentration (%)	<i>Piper jacquemontianum</i>		<i>Piper marginatum</i>	
	24 h.	48 h.	24 h.	48 h.
2.0	55±6.7 a ¹	59±5.5 a	50±4.5 a	55±4.2 a
1.0	37±5.6 b	48±3.9 b	48±3.7 a	49±6.4 b
0.5	21±4.6 c	23±4.2 c	29±5.3 b	34±5.2 c
0.25	18±4.4 d	20±4.9 c	14±5.9 c	15±5.2 d
Tween	0.00 e	0.00 d	0.00 d	0.00 e
H ₂ O	0.00 e	0.00 d	0.00 d	0.00 e
H	62.582	60.765	56.814	55.441
p-value	< 0.0001	< 0.0001	< 0.0001	< 0.0001
LC ₅₀	3.037	1.1666	2.171	1.935
CI	2.78-3.28	1.74-1.87	2.12-2.21	1.90-1.96

Means indicated by the same lowercase superscripts, are different among themselves. LC₅₀=Lethal concentration (50%). CI = 95% Confidence Interval.

The concentration of 2.0% of EOs from two *Piper* species evaluated exhibited higher rate of inviable eggs parasitized by *T. podisi*, 48 hours after the treatment by immersion (Table 3). However the concentrations of 0.25 and 0.50 % did not affect the development of parasitoid *T. podisi* in function of lower concentrations, which represent lower toxic effect on embryo [10]. The exposition time of the treatments evaluated is directly related to the absorption of EO components into the interior of unparasitized and parasitized eggs of *O. insularis* by *T. podisi* by microscopic pores and micropyle, causing their inviability. The reduction in the respiratory rate after 48 hours of treatment is due to the inhibition of oxygen transport towards the interior of the egg of *O. insularis* parasitized by *T. podisi* (Table 3). The variability of results may be due to the effects of different components of EOs from *P. jacquemontianum* and *P. marginatum*, on the embryo of insect-pest and the parasitoid. The ovicidal activity of the EOs both in the embryo of *O. insularis* and *T. podisi* is due to the interaction of EOs components. This may explain the variation in the results shown in Tables 2 and 3, where the LC₅₀ reduced in function of the *Piper* species evaluated and the time of exposure.

Experimental

The essential oils were obtained by hydrodistillation of leaves *P. jacquemontianum* and *P. marginatum* using a Cleavenger type of apparatus [12]. Plants were collected from Soberania National Park and Ocu, Herrera, Panama. Their taxonomic identity was

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established by Alex Espinosa, Taxonomist of CIFLORPAN. The voucher specimens were deposited at the Herbarium of the University of Panama (PMA). The components of EOs were identified by GC MS and published earlier [8,9]. The same essential oils were used for this study as for their chemical composition. reported earlier.

Obtention of parasitized and unparasitized eggs of *Oebalus insularis* by *Telenomus podisi*: The eggs of *O. insularis*, were obtained in controlled abiotic conditions in regulated climatized chambers at 28°C, 80% R.H. and 12 hours of photophase, from adults reared and fed on artificial diet and were submitted to *T. podisi* parasitism, during 24 hours [13].

Application of essential oils to the parasitized and unparasitized eggs of *O. insularis* by *T. podisi*: The egg masses impregnated with EOs at concentrations evaluated were placed in Petri dishes with filter paper at the base, and later transferred to regulated climatized chambers at 28°C, 80% R.H. and 12 hours of photophase. The applications of the EOs by the immersion method were made 24 and 48 hours and the evaluation of the unparasitized eggs of *O. insularis* was made 4 days after the treatment (DAT) (Table 2) and in the parasitized eggs by *T. podisi*, the observation of the inviable eggs parasitized were 10 DAT (Table 3). The dark coloration of unparasitized eggs indicated the death of embryos for their inviability. The inviability of parasitized eggs was determined by the non-emergence of *T. podisi* adults. In each of the concentrations evaluated, as well as in the relative (Tween) and absolute (H₂O) treatments, at different periods of application of the EOs (24 and 48 hours), two hundred unparasitized and parasitized *O. insularis* eggs, in 5 replicates per treatment were used.

Experimental design and statistical analysis: The experimental design was completely randomized. The treatments were analyzed by a Two-Way ANOVA and later by Tukey test at P<0.01. The LC₅₀ was calculated by Probit analysis.

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