Full Length Research Paper

Toxicity and repellency of plant extracts on *Thaumastocoris peregrinus* (Carpintero & Dellapé) (Hemiptera: Thaumastocoridae)

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The eucalyptus bronze bug, *Thaumastocoris peregrinus*, is an exotic pest of eucalyptus crops that has spread worldwide. The objective of this study was to evaluate the toxicity of aqueous plant extracts at 5% of *Matricaria chamomilla*, *Echinodorus grandiflorus*, *Punica granatum*, *Maytenus ilicifolia* and *Origanum majorana* on *T. peregrinus*. For this, choice and no-choice tests were performed. Eucalyptus leaf disks were treated with 5% aqueous extract and two experiments were conducted: (1) the leaf disks were put inside a tube with *T. peregrinus* adults and their longevity was evaluated. Each repetition was one leaf disk/tube (no-choice test); (2) one leaf disk of each treatment was put inside a Petri dish, and offered to *T. peregrinus.* Faecal deposits on each leaf disk were quantified (choice test). In addition, high performance liquid chromatography (HPLC) was carried out to verify phenolic compounds present in the plant extracts. All plant extracts reduce the survival of *T. peregrinus* adults up to nearly 50%. Regarding the choice experiment, *T. peregrinus* fed with eucalyptus disk leaves containing *E. grandiflorus*, *M. chamomilla* and *Maytenus ilicifolia* extracts produced less faecal deposits when compared wiht the other plant extracts and the control group. In addition, HPLC detected gallic, ferulic, caffeic, coumaric and vanillic acid in the extracts samples. These results suggest that these three plant extracts had a repellent effect on *T. peregrinus* adults, aside from reducing its survival, and the phenolic compounds may have contributed to these results.

Key words: Bronze bug, phenolic compounds, *Eucalyptus*.

INTRODUCTION

Thaumastocoris peregrinus (Carpintero and Dellapé) (Hemiptera: Thaumastocoridae), known as the bronze bug, is a small insect from Australia (Carpintero and Dellape, 2006; Noack et al., 2011; Nadel and Noack,

2012). It has spread globally and has become a major pest in various species of eucalyptus in Africa, South America, Europe and New Zealand (Martínez and Bianchi, 2010; Nadel et al., 2010; Wilcken et al., 2010; Laudonia and Sasso, 2012; Sopow et al., 2012).

T. peregrinus feeds on the floem-sap of eucalyptus leaves, causing clorosis and defoliation. Heavy feeding causes reddening of the canopy leaves, known as "winter bronzing". Severe infestations may lead to canopy thinning and decreased tree growth due to the reduced photosynthetic area (Nadel et al., 2010; Wilcken et al., 2010). This insect reaches not more than 4 mm when in adult stage. It has a short life cycle (an average of 35 days) and the female can lay up to 60 eggs during her lifespan (Jacobs and Neser, 2005; Noack and Rose, 2007; Soliman et al., 2012). This high biotic potential enables *T. peregrinus* to have several generations per year, and to have the potential to spread and rapidly establish into new environments (Nadel et al., 2015; Saavedra et al., 2015).

Chemical control has been proven to be effective in urban areas in Australia (Noack et al., 2009), although it raises issues about potential environmental problems. Biological control strategies are being studied to manage *T. peregrinus* population. To date, the egg-parasitic wasp *Cleruchoides noackae* Lin and Hubner (Hymenoptera: Mymaridae) is the most promising, though there are no available published data to confirm its efficiency (Barbosa et al., 2010; Mascarin et al., 2012; Garcia et al., 2013; Santadino et al., 2013; Dias et al., 2014).
Plants with insecticidal activity could also be a viable alternative to control *T. peregrinus*. Botanicals are having renewed importance, due to their eco-toxicological properties and for being a source of bioactive compounds (Zoubiri and Baaliouamer, 2014).

Insecticidal plants have several effects. When not leading to insect mortality, it may cause repellency, deterrence, deformation in pupae and adults, reduce intestinal motility, interfere in the synthesis of ecdysone and chitin (Schmutterer, 1990), growth rate (Nathan et al., 2008), life span and fecundity (Isikber et al., 2006).
Most of the studies that verified these effects on insects have been carried out on disease vectors and agricultural pests. Research confirming insecticidal plants efficiency to control forest pests have been performed (Kanat and Alma, 2004; Sharma et al., 2006; Parel et al., 2014) but no information is available regarding *T. peregrinus.*

Therefore, the objective of this study was to evaluate the toxicity of the aqueous extracts of *Matricaria chamomilla* (Asteraceae), *Echinodorus grandiflorus* (Alismataceae), *Punica granatum* (Punicaceae), *Maytenus ilicifolia* (Celastraceae) and *Origanum majorana* (Lamiaceae) on *T. peregrinus* in the laboratory.

MATERIALS AND METHODS

The bioassays and chemical analysis of the plant extracts

components (high performance liquid chromatography - HPLC) were performed at the Laboratory of Biological Control, and Central of Analysis of the Federal University of Technology - PR, in Dois Vizinhos and Pato Branco (Parana State, Brazil), respectively.

Insects

T. peregrinus eggs were obtained from a well-established colony kept at the Laboratory of Forest Entomology (Embrapa Florestas, Brazilian Corporation of Agricultura Research), reared on *Eucalyptus bentamii* Maiden et Cambage (Myrtaceae) (23 ± 2°C, 60 ± 10% RH and 12 h photoperiod) as described by Beltramin (2014) and the experiments were run under the same conditions.

Nymphs and adults of *T. peregrinus* were reared in branches of *E. benthamii* (23 ± 2°C, 60 ± 10% RH and 12 h photoperiod) and strips of paper towel were distributed along the leaves for oviposition. Strips were checked for eggs daily and were replaced after the eggs collection. Eggs recovered were used in the experiments.

Plant extracts

Leaves of *E. grandiflorus* and *P. granatum* (collected in Dois Vizinhos, Parana State, Brazil and voucher specimens deposited at the Herbarium of the Federal University of Technology – Paraná, UTFPR), *Maytenus ilicifolia, O. majorana* and flowers of *M. chamomilla* (acquired from COOPERFLORA, Turvo, PR) were used to prepare the extracts.

Plant material was dried in oven (60°C for 48 h) and ground in a Willy mill lab grinder. Each of the five plant extracts was prepared at a concentration of 5% w/v by adding 5 g of the plant powder to 100 mL of distilled water and the mixture was kept away from the light from 48 h. Filtration was performed with filter paper (grade 1 : 11 μm), shortly before the start of the experiment. This high concentration was chosen as a standard concentration to be sure if the aqueous plant extracts would affect the insects.

Toxicity (no-choice test)

Fully expanded leaves of *E. dunni* Maiden were washed in sodium hypochlorite 2%, dried and immersed for 5 s in the plant extracts. The control group was immersed in sterile distilled water. After that, the leaves were left to dry in a laminar flow cabinet (5 min, 23°C). Circles of 2.4 cm in diameter were cut from the leaves with a circle cutter near the petiole and put inside sterile flat bottom glass tubes (10 x 9 mm) with hydrogel. One *T. peregrinus* adult (< 2 days old) was placed on each disk leaf. To prevent scape, each tube was covered with voile. One tube was considered a replicate. A total of 12 replicates were conducted per each treatment.

The bioassay was kept in a germination chamber (26 ± 2°C, 60 ± 10% RH and 12 h photoperiod) and *T. peregrinus* survival time was evaluated every six hours, for 144 h. The experimental design was totally randomized and the obtained data was submitted to one-way ANOVA followed by Scott-Knott Test ($p < 0.05$) (Assistat 7.7[®], Silva, 2014).

Repellence activity (free-choice test)

Leaf disks, one from each treatment, were prepared as described above, and put inside glass Petri dishes (150 x 20 mm) lined with filter paper (grade 1 : 11 μm) dampened with water, randomly, at the same distance from each other. In the centre of each disk, 10 adults (< 2 days old) were placed. The dishes were closed and kept in germination chamber (26 ± 2°C, 60 ± 10% RH, 12 h

Table 1. Chromatographic parameters of phenolic compounds from the 5% (w/v) aqueous plant extracts analysed by HPLC.

Phenolic compound	R.T. (min)	UV band (nm)	Linear equation	R^2	LOD ($\mu g.mL^{-1}$)	LOQ ($\mu g.mL^{-1}$)
Gallic acid	8.6	272	Y= 0.313 X – 0.017	0.996	0.10	0.34
Vanillic acid	24.3	260, 280	Y = 0.263 X + 0.023	0.998	0.81	2.72
Caffeic acid	24.6	323	Y = 0.691 X – 0.001	0.997	0.03	0.11
Coumaric acid	28.7	309	Y = 0.540 X – 0.360	0.999	0.02	0.08
Ferulic acid	29.5	322	Y = 0.657 X + 0.007	0.999	0.10	0.36

R.T: Retention time; LOD: detection limit; LOQ: quantification limit.

Table 2. Mean survival time, in hours (± SE) of Thaumastocoris peregrinus confined with Eucalyptus dunni leaves treated with 5% (w/v) aqueous plant extracts under laboratory conditions.

Treatment	Survival (hours)	Survival (days)
Control group	133.0 ± 7.41 [a]	5.5
Punica granatum	98.0 ± 7.91 [b]	4.0
Maytenus ilicifolia	89.0 ± 10.82 [b]	3.7
Echinodorus grandiflorus	77.0 ± 10.22 [b]	3.2
Origanum majorana	70.0 ± 8.90 [b]	2.9
Matricaria chamomilla	68.5 ± 6.66 [b]	2.8
F	7,1024**	

*Means followed by the same letter do not differ by Scott-Knott Test (p < 0.05).

photoperiod), and the number of faecal drops on each leaf disc was evaluated every 24 h for seven days (daily faecal drops (DFD) = total of faecal drops (TFD) – previous day total of faecal drops (PFD)).

Each dish was considered a repetition and 24 repetitions were used for this experiment. The experimental design was split-plot and the data was submitted to ANOVA followed by Tukey Test (p < 0.05) (Assistat 7.7®, Silva, 2014).

Qualitative analysis of high performance liquid chromatography (HPLC) profiling

The qualitative analysis of extracts compounds was carried out using reversed-phase HPLC (Francisco and Ressurreccion, 2009) with slight modifications. Each aqueous extract sample (10 µL) was injected in an HPLC equipment with photodiode array and fluorescence detection, reverse-phase column C18 (250 x 4.6 mm, 5 m). The mobile phase consisted of water/acetic acid (0.1%, v/v) (solvent A) and methanol/acetic acid (0.1% v/v) (solvent B), at 1 mL/min. The gradient conditions were as follows: 5-7% solvent B in 7 min, 17% of B in 75 min, 45% of B in 110 min, 70% of B in 117 min, 100% of B in 124 min and 5% of B in 129 min. The column was kept at 30°C and the chromatograms processed with Galaxie™ software.

Identification of unknown compounds was based on matching their retention times with those of pure standards and by the absorption spectra at the wavelengths 272, 313 and 360 nm of the ultraviolet region with photodiode detector. Quantification was determined with external standardization with identical standards of ferulic, gallic, vanillic, caffeic and coumaric acid in concentrations from 0.5 to 7.5 $\mu g.mL^{-1}$ (Table 1). The detection limit (DOL) and

quantification limit (QOL) values were obtained with the calibration curve equation according to ICH (1996).

RESULTS

All plant extracts affected *T. peregrinus* adults, reducing their survival, when compared with the control group (Table 2). Analysis of faecal deposits in the free-choice bioassay, showed that they did not differ significantly between treatments at 24, 48, or 144 h after starting the experiment. Nevertheless, *T. peregrinus* fed with eucalyptus disk leaves containing *E. grandiflorus*, *M. chamomilla* and *Maytenus ilicifolia* extracts produced less faecal deposits when compared with the other plant extracts and the control group (Table 3).

High performance liquid chromatography

The phenolic compounds, gallic, vanillic, caffeic, coumaric and ferulic acid were identified and quantified from the plant extracts (Table 4). The highest and lowest concentration of gallic acid was found on *P. granatum* (1.84 $mg.g^{-1}$) and *O. majorana* (0.72 $mg.g^{-1}$), respectively. Vanillic acid was found only in *Maytenus ilicifolia* (0.35 $mg.g^{-1}$). Caffeic acid was detected in both *M. chamomilla*

Table 3. Mean of *Thaumastocoris peregrinus* faecal drops (± SE) in *Eucalyptus dunni* disk leaves treated with 5% (w/v) aqueous plant extracts in a free-choice trial, under laboratory conditions.*

Treatments	Number of faecal drops over time (hrs)						
	24	48	72	96	120	144	168
Control group	2.87 ± 0.98 ns	1.04 ± 2.47 ns	22.88 ± 2.70 [a]	27.58 ± 3.49 [a]	29.54 ± 3.49 [a]	29.13 ± 3.36 ns	34.33 ± 4.01 [a]
Punica granatum	1.58 ± 0.41	6.63 ± 1.19	14.42 ± 1.81 [ab]	19.00 ± 1.613 [ab]	23.67 ± 1.56 [ab]	26.04 ± 1.54	30.87 ± 1,17 [ab]
Maytenus ilicifolia	2.00 ± 0.47	8.00 ± 1.51	11.08 ± 1.93 [b]	13.96 ± 2.25 [b]	18.17 ± 2.61 [b]	21.79 ± 2.44	27.66 ± 2.76 [ab]
Echinodorus grandiflorus	1.25 ± 0.52	8.08 ± 1.83	12.17 ± 2.56 [b]	16.04 ± 2.84 [b]	18.46 ± 3.14 [b]	22.63 ± 3.36	25.12 ± 3.42 [ab]
Origanum majorana	3.16 ± 0.64	12.00 ± 1.87	15.96 ± 2.22 [ab]	20.33 ± 2.40 [ab]	25.63 ± 3.16 [ab]	29.88 ± 3.36	33.97 ± 3,41 [a]
Matricaria chamomilla	1.87 ± 0.61	8.42 ± 1.93	12.42 ± 2.47 [b]	15.75 ± 2.90 [b]	19.67 ± 3.01 [ab]	21.97 ± 3.05	23.54 ± 3.16 [b]
Dms	10068						
F	1.9875*						

*Means followed by different letters in the same column differ significantly according to Tukey Test ($p < 0.05$); ns – not significant.

Table 4. Relative concentrations of phenolic compounds obtained from 5% (w/v) aqueous plant extracts.

Extract	Concentration mg.g^{-1}				
	Gallic acid	Vanillic acid	Caffeic acid	Coumaric acid	Ferulic acid
Punica granatum	1.84	n.d	n.d	n.d	n.d
Maytenus ilicifolia	n.d	0.35	0.06	n.d	n.d
Echinodorus grandiflorus	0.30	n.d	n.d	0.01	0.46
Origanum majorana	0.72	n.d	n.d	n.d	n.d
Matricaria chamomilla	0.23	n.d	0.72	n.d	1.41

n.d.- Not discovered.

and *M. ilicifolia* (0.72 mg.g^{-1} and 0.06 mg.g^{-1}), respectively. Coumaric acid was only found in *E. grandiflorus* (0.01 mg.g^{-1}). Ferullic acid was found in both *M. chamomilla* (1.41 mg.g^{-1}) and *E. grandiflorus* (0.46 mg.g^{-1}).

DISCUSSION

The plant extracts from *P. granatum*, *O. majorana*, *M. chamomilla*, *M. ilicifolia* and *E. grandiflorus* reduced survival of *T. peregrinus* adults. The leaf disks treated with the last three extracts presented a reduced number of faecal drops, indicating a repellent/deterrent effect.

The presence of phenolic compounds might have had an important role in these results. Phenolic compounds are generally regarded as antifeedants, digestibility reducers and toxic to insects (Rani and Pratyusha, 2013). The same compounds found in this research have been encountered in elevated levels in rice after these plants were attacked by insect pests, indicating their production as a result of induced plant defence (Rani and Jyothsna, 2010), corroborating with our findings.

Assays regarding the effect of plant extracts on heteropterans have been carried out with other plants, other forms of extraction and other insects (Carneiro et al., 2013; González et al., 2011;

Krinski and Massaroli, 2014). Essential oils were tested on the behaviour of *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae), indicating repellent, irritant and toxic effects (Emilie et al., 2015), and *Mentha piperita* L. demonstrated repellent and insecticidal activity against *Brevicoryne brassicae* L. (Homoptera: Aphididae) (Wubie et al., 2014); but there was no research on the group Thaumastocoridae.

Phenols like coumaric and ferulic acid, found in the plant extracts used in our bioassays, may be found in the insoluble or cell wall, as reservoir for lignin biosynthesis, which by itself may be a defence mechanism (Lattanzio et al., 2006). In addition to toxic and deterrent action of phenolic compounds, oxidation of phenols to polymers, catalysed by polyphenol oxidase (PPO) and peroxidase (POD) is another potential defence mechanism in plants against herbivorous insects, which reduce digestibility, palatability and nutritional value. Quinones formed by oxidation of phenols, for instance, bind covalently to leaf proteins and inhibit the protein digestion in herbivores (Bhonwong et al., 2009). *Artemisia annua* L. (Asteraceae), for example, reduces digestive enzymes activity in *Eurygaster integriceps* Puton (Heteroptera: Scutelleridae) (Zibaee and Bandani, 2010).

Quinones also exhibit direct toxicity on insects (Duffey and Stout, 1996; Bhonwong et al., 2009). Alkylation of amino acids reduces the nutritional value of plant proteins for insects, which in turn negatively affects the insect growth and development (Bhonwong et al., 2009). Phenols also play an important role in cyclic reduction of reactive oxygen species (ROS) such as superoxide anion and hydroxide radicals, which in turn activate a cascade of reactions leading to the activation of defensive enzymes (Maffei et al., 2007).

Thus, the phenolic compounds present in *M. chamomilla*, *E. grandiflorus*, *M. ilicifolia*, *O. majorana* and *P. granatum* extracts may have played an important role in the obtained results of this experiments, both in repellent and toxic effect.

These results represent basic research, consequently they should be used to help select plants with insecticidal/repellent properties; and from there, obtain extracts by different extraction methods, detect the active compounds responsible for the action on the insects, and develop environmental-friendly insecticides.

Conflict of Interests

The authors have not declared any conflict of interests.

ACKNOWLEDGEMENTS

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