Insecticidal activity of essential oil from *Eucalyptus globulus* against *Aphis nerii* (Boyer) and *Gynaikothrips ficorum* (Marchal)

Russo, S., Yaber Grass, M. A., Fontana, H. C. and Leonelli, E.

SUMMARY

Aphids and thrips are very important pest species of many greenhouse and field plants in the world. In recent years, there have been attempts to identify plants for their insecticidal property with a view to find out suitable alternatives to the indiscriminate use of chemical pesticides, which are a risk for human health and the environment. The aim of this work was to evaluate the insecticidal effect of *Eucalyptus globulus* Labill essential oil (EO) against *Aphis nerii* Boyer de Fonscolombe (Hemiptera: Aphididae) and *Gynaikothrips ficorum* Marchal (Thysanoptera: Ploeothridae). The EO was extracted by hydrodistillation and the bioassay was evaluated by toxicity contact method, using solutions at different concentrations and for different time intervals. The EO was more toxic to *G. ficorum* (LC_{50} = 0.031µL cm^{-2}) than *A. nerii* (LC_{50} = 0.099µL cm^{-2}) on filter discs at 12 h of exposure. These results suggested that EO from *E. globulus* constitute a good alternative for the control of *A. nerii* and *G. ficorum*, considering their insecticidal effects at low applied concentrations and for short periods of time.

Keywords: secondary metabolites, thrips, aphids, *Eucalyptus globulus.*

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RESUMEN

Los áfidos y trips son dos de las más importantes especies de plagas que invaden invernaderos y cultivos en el mundo. En los últimos años se han realizado muchos esfuerzos con el propósito de identificar plantas por sus propiedades insecticidas, como una alternativa al uso indiscriminado de insecticidas químicos que constituyen un riesgo para la salud humana y el medioambiente. El objetivo de este trabajo fue evaluar el efecto insecticida del aceite esencial (AE) de *Eucalyptus globulus* Labill sobre *Aphis nerii* Boyer de Fonscolombe (Hemiptera: Aphididae) y *Gynaikothrips ficorum* Marchal (Thysanoptera: Ploeothridae). El AE se extrajo por hidrodestilación y

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los bioensayos se evaluaron usando el método de toxicidad por contacto a diferentes concentraciones e intervalos de tiempo. Estudios exploratorios se realizaron previamente para determinar las concentraciones apropiadas de testeo. El AE fue más tóxico sobre *G. ficorum* (LC_{50} = 0.031µL cm^{-2}) que sobre *A. nerii* (LC_{50} = 0.099µL cm^{-2}) en el ensayo realizado en discos de papel de filtro correspondiente a las 12 h de exposición. Estos resultados sugieren que el AE de *E. globulus* constituye una buena alternativa para el control de *A. nerii* y *G. ficorum* considerando el efecto insecticida a bajas concentraciones y cortos tiempos de exposición.

Palabras clave: metabolitos secundarios, trips, áfidos, *Eucalyptus globulus.*

Russo, S., Yaber Grass, M. A., Fontana, H. C. and Leonelli, E.: Universidad de Buenos Aires, Facultad de Agronomía. Avenida San Martín 4453, 1417, Ciudad Autónoma de Buenos Aires, Argentina. Correspondence to: srusso@agro.uba.ar

INTRODUCTION

The recent migrations from villages to urban cities have resulted in an increase of the number of green spaces for the family recreation and frequently we can encounter species of ornamental plants cultivated in them. Several harmful factors damage plants and one of the most important is the presence of insects (Kaplanski and Venier, 2012).

This study focuses on *Aphis nerii* Boyer de Fonscolombe (Hemiptera: Aphididae) and *Gynaikothrips ficorum* (Marchal) (Thysanoptera: Ploeothridae). This aphid is one of the common pests of several important ornamental plants, its host range includes several genera of Asclepiadaceae such as *Morrenia*, *Asclepias curassavica* L, *Ceropegia woodii* Schltr. and Apocynaceae (*Nerium* and *Vinca*) (Mohammad, Mohammad and Salma, 2012). As regards the thrip under study, it comprises approximately 40 species of dark brown to black thrips which induce galls on developing leaf tissues of *Ficus* species (Rosales: Moraceae) (Dang, Mound and Qiao, 2014). *Ficus* spp. is one of the most common and widespread tropical genus in the modem era. Especially, *Ficus benjamina* L., these ornamental and urban trees, are widely disseminated in several countries and especially in Argentina (Frodin, 2004; De Borbón and Agostini, 2011).

Aphids and thrips are serious pests and vectors that transmit viruses to field crops, vegetables, ornamentals, and greenhouse crops. They have displayed a remarkable ability to develop resistance to almost every insecticide with which they have been treated (Arthurs, Guixin Chen and Chen, 2016).

Eucalyptus globulus (Myrtaceae) is commonly grown in central areas of Argentina, its leaves contain compounds such as α-pinene and β-pinene, globulol, terpineol, and specially 1,8-cineole, the most predominant monoterpene (Lucia, Licastro, Zerba, Audino and Masuh, 2009). These secondary metabolites demonstrated insecticidal and repellent properties against pests in stored grains (Russo, Cabrera, Chludil, Yaber Grass and Leicach, 2015).

The application of chemical pesticides and fumigants is the most economical and common practice used but it has disadvantages that have been reported in several countries. They have reported the development of insect strains resistant to pesticides, the presence of residues toxic for human consumption, acute and chronic toxicity for workers and adverse effects on the environment. (Elumalai, Krishnappa, Anandan, Govindarajan and Mathivanan, 2010; Boyer, Zhang and Lempèriere, 2012). Studies indicate that plant secondary metabolites, may affect significantly the plant resistance to parasites and microorganisms (Pérez-Urria Carril and Ávalos García, 2009; Sepúlveda Jiménez, Porta-Ducoing and Rocha-Sosa, 2004).

Taking into account this situation, botanical insecticides are a sustainable alternative to integrated pest management that could contribute to reducing the use of synthetic insecticides. This work reports on *E. globulus* EO insecticidal activity against A*. nerii* and *G. ficorum* adults.

MATERIAL AND METHODS

Essential Oil Extraction

Fresh mature leaves of *E. globulus* were collected from plants cultivated in Lobería, Buenos

Aires, Argentina (38º 9' 48'' S, 58º 46' 53'' W). The EO was extracted from freshly collected leaves (150 g) by hydrodistillation using a modified Clevenger-type apparatus for 2 h and stored at -18 ºC. Essential oil content was calculated for each repetition (n = 4) and expressed as a percentage (v/w, fresh weight). Phytochemical data analyses were performed by gas chromatography (GC) and gas chromatography–mass spectrometry (GC-MS) and were reported in a previous work (Russo *et al.*, 2015).

Insecticidal activity bioassay

Experiments were performed in the Laboratory of Agricultural Zoology (Agronomy School, University of Buenos Aires). *Aphis nerii* and *Gynaikothrips ficorum* adult insects were collected in the campus of the Agronomy School. The insects used for bioassays were taken directly from the field and they have not been reared in the laboratory because of the reduced information about nutritional factors, and climatic requirements of the species biological cycle. The insecticidal activity of *E. globulus* EO was evaluated by contact method conducted using filter paper discs (Whatman nº 1, 9-cm diameter pieces). Range-finding studies were run to determine the appropriate testing concentrations for each insect. EO samples were dissolved in acetone and applied on the surface of the paper in an amount equal to 0, 0.05, 0.10, 0.15 and 0.20 µL cm^{-2} for *A. nerii* and 0, 0.10, 0.15, 0.20 and 0.25µL cm^{-2} for *G. ficorum*. After 10 min when the solvent had evaporated, 10 adult aphids and thrips were deposited into each glass Petri dish. Four replicates were made for each treatment and a control using acetone. Mortality percentages were determined after 0.5, 2, 4, 6, 12, 24 and 48 h-insects were considered dead when, prodded with a fine brush (maximum three times), they showed no appendage movement. Mortality was determined by the percentage of dead insects after the time of exposure to EO concentration. Percent mortality values were computed using the formula: M (%) = (n/N) x 100; where: n = the number of dead insects, and N = the number of insects on treated discs. Mortality data were subjected to probit analysis to determine the lethal concentration 50 (LC_{50}) values (with 95% confidence limits).

Statistical analysis

Statistical methods were employed using InfoStat/Professional software version 1.1 (Di Rienzo *et. al,* 2002). Tests of normality (Kolmogorov-Smirnov test) and homogeneity of variance (Levene test) were applied in each analysis. Mortality and EO concentration over the analyzed periods were performed using two-way ANOVA, and differences between means were compared to the control using Tukey's test with a 0.05 confidence interval in a completely randomized design. Different capital letters indicate significant differences between values in the same row, and different small letters indicate significant differences between values in the same column. To estimate LC_{50} values, mortality data were subjected to Probit analysis (Finney, 1971, 2nd edition). Results from each program were pooled and statistical analysis conducted to find regression equation (Y = mortality; X = concentrations), using GraphPad Prism 6.01 (GraphPad Software, 2012).

RESULTS AND DISCUSSION

The toxicity bioassay indicated that EO showed insecticidal properties against *A. nerii* adults (Table 1). After 30 min of exposure, the treatment with 0.20µL cm^{-2} EO against *A. nerii* presented 35% of dead adults, which is the highest value for this time of exposure. The same mortality rate was observed for the lowest assayed concentration (0.05µL cm^{-2}) but only at 24 h. The two EO treatments with the

Table 1. Concentrations, exposure time and mortality of essential oil from *Eucalyptus globulus* in adult *Aphis nerii*

	Control	**0.05µL cm⁻²**	**0.10µL cm⁻²**	**0.15µL cm⁻²**	**0.20µL cm⁻²**
M (0.5 h)	0**A**	0**Aa**	0**Aa**	7.50 ± 1.52**Ab**	35.00 ±5.77 **Eg**
M (2 h)	0**A**	7.50 ± 2.50**Ab**	7.50 ± 2.00**Ab**	10.00 ± 1.16**Abc**	40.00 ± 8.16**Eh**
M (4 h)	0**A**	7.50 ± 2.50**Ab**	10.00 ±1.16**Abc**	20.00 ±2.16**Ae**	60.00 ± 8.16**Hi**
M (6 h)	0**A**	7.50 ± 2.50**Ab**	12.50 ± 1.**57A-d**	32.50 ± 2.15**Dg**	87.50 ± 12.58**J-l**
M (12 h)	0**A**	30.00 ± 2.16**B-g**	32.5 ± 2.57**B-f**	77.50 ± 3.50**Ijk**	95.00 ± 10**Kl**
M (24 h)	0**A**	32.50 ±2.57**D-g**	45.00 ± 4.77**Gh**	95.00 ± 5.77**Kk**	100.00 ± 0.00**Ll**
M (48 h)	0**A**	42.50 ± 3.50**F-h**	72.50 ± 5.57**Ij**	100.00 ± 0.00**Ll**	100.00 ± 0.00**Ll**

M: adult mortality mean values (%, n = 4). Different letters indicate significant differences verified by ANOVA two-way and Tukey's test (P<0.05) Capital letters represent comparisons between the applied concentrations (in columns rows), and lower case letters represent comparisons between the observation times (in rows columns).

highest concentrations (0.15 and 0.20μL cm^{-2}) exhibited the death of all insects when the exposure time was 48 and 24 h, respectively.

The results suggested that, at 24 h of exposure, EO applied at 0.15μL cm^{-2} presented effective activity with values under 100% (95%) up to 100% after 48 h.

Eucalyptus EO at the lowest tested concentration (0.10μL cm^{-2}) against adult *G. ficorum* (Table 2) had noticeable insecticidal effects because the mortality achieved was of 55% in 6 h and 100% at the end of experiment. EO at 0.15μL cm^{-2} achieved the maximum lethal effect when the bioassay was concluded (24 h).

After recalculating statistical significance between the different applied concentrations for each exposure time, we observed that at 12 h all EO solutions showed the most significant differences with respect to the control (Tables 1 and 2). At this time, the highest concentrations (0.25μL cm^{-2}) achieved 100% of mortality. In view of the above, mortality data at 12 h were subjected to probit analysis to determine the lethal concentration 50 (LC_{50}) values (with 95% confidence limits). From the probit analyses, the calculated regression line equation was Y = 25,02*X + 2,515 and Y = 11,74*X + 4,628 for *A. nerii* and *G. ficorum* respectively. According to these results, *G. ficorum* adults (LC_{50} = 0.031 ± 0.01μL cm^{-2}) were more sensitive than *A. nerii* adults (LC_{50} = 0.099 ± 0.01μL cm^{-2}) to *E. globulus* EO.

Similar observations were found by Regnault-Roger, Philogene and Vincent (2004) and Russo *et al.* (2015). They demonstrated that *E. globulus* EO had insecticidal effects against adult insects of stored grains: *Acanthoscelides obtectus* (Coleoptera: Bruchidae) and *Tribolium confusum* (Coleoptera: Tenebrionidae). Additionally, Mareggiani, Russo and Rocca (2008) and Castresan, Rosenbaum and González (2013) reported changes of behavior patterns and lethal effects in A. *gossypii* and *Macrosiphum euphorbiae* aphids when they were treated with *E. globulus* EO. Several investigations were carried out on the insecticidal effects of *E. globulus* EO against different insects (Russo *et al.*, 2015; Kumar, Mishra, Malik and Satya, 2012). The bioassay carried out by Mossi *et al.* (2011) also demonstrated insecticidal activity of *E. globulus* EO against *S. zeamais*, the major pest of maize. They carried out the assay on filter paper and informed that EO caused 100% mortality after 24 h of exposure at concentrations equal to 0.16μL cm^{-2} and a LC_{50} value of 0.10μL cm^{-2}.

These results suggest that *E. globulus* EO has effective insecticidal activity in a concentration of 0.15μL cm^{-2} against *A. nerii* and *G. ficorum*, because the mortality percentage of adults reached 95% after 24 and 12 hours of exposure time, respectively. EO from *E. globulus* constitutes a good alternative to the control of *A. nerii* and *G. ficorum*, considering its insecticidal effects at low applied concentrations and short exposure times. However, this preliminary laboratory investigation could represent the basis for further field research on the efficacy and the viability of the application of this EO against aphids and thrips.

CONCLUSIONS

In conclusion, *E. globulus* EO had valuable bio-insecticidal effects at low concentrations and short exposure times against *A. nerii* and *G. ficorum*. In the context of integrated pest management, due to its low toxicity, rapid degradation in the environment and efficiency in pest control, it provides a friendly alternative to chemical insecticides.

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Table 2. Concentrations, exposure time and mortality of essential oil from *Eucalyptus globulus* in *Gynaikothrips ficorum*

	Control	0.10μL cm^{-2}	0.15μL cm^{-2}	0.20μL cm^{-2}	0.25μL cm^{-2}
M (0.5 h)	0**A**	0**A**	10.00 ± 0.00**Ab**	20.00 ± 14.14**Ac**	22.50 ± 2.00**Bc**
M (2 h)	0**A**	25.00 ± 2.77**Bc**	25.00 ± 1.77**Bc**	30.00 ± 2.16**Cd**	50.00 ± 1.50**E**
M (4 h)	0**A**	45.00 ± 2.77**De**	47.50 ± 2.57**E**	60.00 ± 3.50**E**	92.50 ± 2.57**Fg**
M (6 h)	0**A**	55.00 ± 2.77**E**	80.00 ± 0.00**F**	85.00 ± 3.77**Fg**	97.50 ± 3.00**G**
M (12 h)	0**A**	90.00 ± 0.00**Fg**	95.00 ± 3.77**Fg**	95.00 ± 2.00**Fg**	100.00 ± 0.00**G**
M (24 h)	0**A**	100.00 ± 0.00**G**	100.00 ± 0.00**G**	100.00 ± 0.00**G**	100.00 ± 0.00**G**

M: adult mortality mean values (%, n = 4). Different letters indicate significant differences verified by ANOVA two-way and Tukey's test (P<0.05) Capital letters represent comparisons between the applied concentrations (in columns rows), and lower case letters represent comparisons between the observation times (in rows columns)

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