**Selectivity and Sublethal Effects of Botanical Extracts to Pupae of *Trichogramma pretiosum* Riley, 1879 (Hymenoptera: Trichogrammatidae)**

Joanina Gladenucci1*, Indiamara Marasca2
José Carlos de Almeida Pernambuco Filho3, Filipe Pereira Giardini Bonfim3
and Regiane Cristina Oliveira de Freitas Bueno3

1Departamento de Entomologia, Universidade Federal de Lavras (UFLA), CEP 37200-900, Minas Gerais, MG, Brasil.
2Universidade Do Rio Verde (UNIRV), Goiás, GO, Brasil.
3Universidade Estadual Paulista (UNESP), São Paulo, SP, Brasil.

**Authors’ contributions**

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

**Article Information**

DOI: 10.9734/JEAJ2020/v42i930594

(1) Dr. Rusu Teodor, University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, Romania.
(2) Ibrahim Abdallah, Cairo University, Egypt.
(2) José-Luis Reyes-Carrillo, Universidad Autónoma Agraria Antonio Narro, Mexico.

Complete Peer review History: http://www.sdiarticle4.com/review-history/62763

Received 14 September 2020
Accepted 21 November 2020
Published 11 December 2020

**ABSTRACT**

**Aims:** assess the effect of botanical extracts on pupae of the natural enemy *Trichogramma pretiosum*.

**Study Design:** evaluation of the selectivity and sublethal effect on *T. pretiosum* pupae.

**Place and Duration of Study:** Departamento de Proteção Vegetal e Horticultura da Faculdade de Ciências Agronômicas – Universidade Estadual Paulista “Júlio de Mesquita Filho” (FCA/UNESP) – Campus de Botucatu, São Paulo, Brazil, carried out between March 2016 and April 2017.

**Methodology:** the experiment was conducted in laboratory, with eleven treatments: *Rosmarinus officinalis*, *Mikania glomerata*, *Varronia curassavica*, *Chenopodium ambrosioides*, *Vermonia polyanthes*, *Plectranthus amboinicus*, *Tetradenia riparia*, *Artemisia absinthium*, *Cymbopogon citratus*, distilled water and chlorpyrifos, with the two last ones being the negative and positive controls.
controls, respectively. The plants were collected in the morning and had 100 g of each species separated and immersed in 300 ml of methyl alcohol for 24 hours in triple extraction. Posteriorly, the solvent was evaporated in rotavapor and the crude extract diluted in the proportion of 1000 mg to 250 mL of distilled water. The parasitism (%) was evaluated. The mortality of the parasitoids was classified in: class 1 – innocuous (E < 30%); class 2 – slightly harmful (30 ≤ E ≤ 79%); class 3 – moderately harmful (80 ≤ E ≤ 99%); class 4 – harmful (E > 99%); daily and accumulated parasitism, total of parasitized eggs and longevity were reported.

Results: Solely the extract of R. officinalis was selective to T. pretiosum pupae. Despite it, all tested extracts induced sublethal effects, with R. officinalis and C. citratus causing the highest damage. Although R. officinalis resulted in sublethal effects, it was the only extract which did not lead to the mortality of females of T. pretiosum.

Conclusion: the tested botanical extracts are innocuous (class 1) or slightly harmful (class 2) to pupae of T. pretiosum, resulting in some sublethal effect that might spoil its efficiency in the Integrated Pest Control.

Keywords: Biologic control; botanical insecticides; egg parasitoid; integrated pest control.

1. INTRODUCTION

Botanical extracts are considered natural products and were intensively used between the 30s and 50s in the control of medical and agricultural pests, being later substituted by synthetic insecticides [1,2]. These extracts contain secondary metabolites produced by plants, which are used as herbivory defense [3] and might be used as a part of the Integrated Pest Control (IPM). However, these compounds may have toxic action on natural enemies and are not always selective.

The term “selectivity” represents the potential of a given product in controlling the pest while exerting little to no effects over the beneficial local entomofauna [4]. The International Organization for Biological Control (IOBC) coordinates studies on selectivity, establishing standardized methodologies for laboratory, semi-field and field experiments. These studies amply use species of the egg parasitoid Trichogramma, as they are easily reared under laboratory conditions while using alternative hosts [5].

At first selectivity characteristics were given to an insecticide by measuring its lethal effect on natural enemies, or in other words, by measuring the mortality of the subjects in the short term. Nonetheless, several of these products may cause sublethal effects, such as reduction of longevity, capability of prey/parasitism, modifications in different levels of the biological or behavioral characteristics, reduction in development and reproduction rates (fecundity, fertility, sexual ratio) and morphological deformations [6-8]. There is a continuous growing demand of these natural products in the market of chemical products made to control pests, as they not only are efficient in controlling a pest population, but also contribute positively to the equilibrium of agroecosystems. In this context, the aim of this work was to assess the selectivity and sublethal effects of botanica extracts on pupae of T. pretiosum.

2. MATERIALS AND METHODS

The experiment was made at the Departamentos de Proteção Vegetal e Horticatura, da Faculdade de Ciências Agronômicas, Universidade Estadual Paulista “Júlio de Mesquita Filho” (FCA/UNESP), Campus de Botucatu, São Paulo, Brazil in controlled conditions (14h of photophase, 25°C and 70% of relative humidity).

2.1 Treatment Selection

The botanical extracts of Rosmarinus officinalis, Mikania glomerata, Varronia curassavica, Chenopodium ambrosioides, Veronica polyanthes, Plectranthus amboinicus, Tetradenia riparia, Artemisia absinthium, Cymbopogon citratus used in this bioassay were selected based on previous results obtained in second instar larvae of Spodoptera frugiperda (J. E. Smith, 1797) (LEPIDOPTERA: NOCTUIDAE), in which the extracts were pulverized in a Potter Tower® (by contact) and also incorporated in the larvae’s artificial diet (ingestion). Thus, the criteria of these species’ selection were insecticidal effect of the plant extracts on S. Frugiperda, in which the concentration was 1000
mg of crude extract to 250 ml of distilled water (GLADENUCCI, 2015). The active ingredient chlorpyrifos, an organophosphorus insecticide commonly used in selectivity tests, was used as positive control (as recommended by the IOBC) and distilled water as negative control.

2.2 Botanical Extracts Preparation

The extracts were obtained in the laboratory of Plantas Medicinais do Departamento de Horticulutra da FCA/UNESP. Leaves were collected during morning and cultivated under organic management of the medicinal garden, then taken to the laboratory. A hundred grams of fresh leaves from each species were macerated in methanol, immersed in 300 ml of methyl alcohol for 24, 48 and 72 hours, in triple extraction, resulting in 900 ml/L of methanolic extract botanical. The solvent was evaporated in a rotary evaporator (Quimis) and the crude extract diluted in the proportion of 1000 mg/ML to 250 ml/L of distilled water and then conserved in a glass recipient with amber coloration.

2.3 Maintenance of the Creation of T. pretiosum

Adults of T. pretiosum were ceded by a biofactory and from there the creation was reared inside four liters recipients and fed with chunks of crude honey. Fresh eggs of Ephestia kuehniella Zeller (LEPIDOPTERA: PYRALIDAE) glued to coated paper by a double-sided adhesive tape were made available for 24 hours to allow parasitism. Afterwards, each paper sheet was individualized in new recipients until the hatching occurred, marking the beginning of a new rearing cycle. The rearing was kept under controlled conditions of temperature (25±2°C), relative humidity (70±10%) and photophase (14 hours).

2.4 Pulverization of Botanical Extracts on Bioassays

The pulverizations were made in the laboratory of Tecnologia de Aplicação, do Departamento de Proteção Vegetal da FCA/UNESP. All the applications were done in a Potter Tower®, set to apply 250 L ha⁻¹ with pressure varying between 5 to 7 psi and deposition of 1.75 ± 0.25 mg cm⁻², measured with an analytical balance, following the standards of the IOBC. For the pulverization, 1.13 ml of each treatment extract were separated in Eppendorf® tubes and then pulverized over the target.

2.5 Cages for Selectivity Assessment

The cages were built as recommended by the IOBC, with aluminum framework juxtaposed with screws. In three sides of the cage, there are ventilation openings which are sealed with voile tissue to prevent the fleeing of the parasitoids but also enable gas exchange [9]. In the fourth side there is an opening used to insert the coated paper with eggs and another opening used for releasing the parasitoids inside the cages. The top and bottom sides are completely sealed with a glass wall and are interconnected by a hose-system connected to a vacuum pump used to remove air by suction.

2.6 Selectivity on Pupae of T. pretiosum

The experimental design was made in randomized blocks, with eleven treatments and five repetitions. Paper cards of 1.5 cm² (± 600 eggs) with parasitized eggs were pulverized with the treatments. The eggs were already parasitized for about 168 to 192 hours, a time period in which the parasitoid already changed from larvae to the pupae stage [10]. Afterwards the pulverization had fully dried in natural conditions, the paper cards were put inside the cages where the parasitoids emerged and fed with chunks of crude honey. In the next 24, 48 and 72 hour time period, paper cards of 6.25 cm² with fresh eggs of E. kuehniella were made available to stimulate parasitism. After 96 hours the cages were disassembled, the paper card with eggs collected and individualized by date in plastic bags to posterior analysis. The parasitism (P) was analyzed with the equation $P = \frac{\text{number of parasitized eggs}}{\text{numbers of offered eggs}} \times 100$. The reduction in the parasitism in relation to the negative control was measured by the equation of efficiency: $E(\%) = (1 - (\text{percentage of the treatment general mean} / \text{percentage of the control treatment general mean})) \times 100$, where: E(%) means the percentage of reduction in the beneficial capability of the parasitoid [11,12]. Thus, the treatments were classified accordingly to their mean of reduction, as recommended by the IOBC: class 1 – innocuous (E < 30%); class 2 – slightly harmful (30 ≤ E ≤ 79%); class 3 – moderately harmful (80 ≤ E ≤ 99%); class 4 – harmful (E > 99%) [13].
2.7 Sublethal Effects Caused by Botanical Extracts on *T. pretiosum*

The experimental design was entirely randomized with 20 repetitions. Stacks of five paper cards of 6.25 cm² (± 1.200 eggs) with parasitized eggs were separated to each treatment, put inside tubes of flattened bottom and sealed with plastic film. At the moment the eggs had 168 to 192 hours of parasitism (same reason as abovementioned in 2.6), the stacks were temporarily removed from the tubes and received a pulverization of its respective treatment, to be later returned to its original recipient, which was sealed again until the parasitoid emerged.

Afterwards emergence, females of *T. pretiosum* were separated (based on antennal features [14] and individualized in Duram® tubes, totalizing 20 females per treatment (20 repetitions). In a daily basis, these females had access to paper cards of 0.25 cm² with fresh eggs of *E. kuehniella* (± 180 eggs) until they died. The paper cards were labeled and stored to posterior analysis. It was analyzed the total of parasitized eggs and the longevity of the parasitoids, although the sublethal effects may affect the insect physiology and interfere in the evaluated parameters [15].

2.8 Statistical Analysis

The results were analyzed with an Analyses of variance (ANOVA) and the means compared with a Turkey’s test with 5% of significance with the software SISVAR 5.6.

3. RESULTS AND DISCUSSION

3.1 Selectivity on Pupae of *T. pretiosum*

The egg parasitoid *T. pretiosum* has its embryonic and post-embryonic development inside the eggs of pest lepidopterans [16]. In our study, the parasitoids received the treatment in the pupae stage, about to emerge, which is marked by the protection of the pupae shell. Aside from this natural protection, the parasitoid is also protected by the host egg’s chorion [17],[18].

Thus, females which emerged from eggs pulverized with water treatment (negative control) did not have any reduction in the parasitism capability and parasitism percentage in the first day (Table 1). At second and third day, as expected, there was reduction in the parasitoid activeness (Tables 2, 3) and despite it, the negative control was classified as innocuous (class 1).

The positive control (chlorpyrifos) caused reduction of the parasitism and hence was classified as moderately harmful (class 3) (Tables 1, 2, 3), indicating that the treatment penetrates de egg’s chorion and reaches the parasitoid system, even with the pupal protection [19].

In the first day, treatments with botanical extracts had little influence over the parasitism and parasitism capability, being classified as innocuous (class 1). The only exception was the treatment made with *A. absinthium*, which was classified as slightly harmful (class 2), as it caused lesser parasitism percentages and reduction in the parasitism capability (Table 1).

At the second day, the extract of *R. officinalis, V. curassavica* and *T. riparia* had little significant difference when compared to the negative control and hence were classified as innocuous (class 1). The extracts of *M. glomerata, C. ambrosioides, V. polyanthes, P. amboinicus, A. absinthium* and *C. citratus* resulted in the reduction of parasitism capability and were classified as slightly harmful (class 2). When compared to the negative control, the treatments with *A. absinthium* and *C. citratus* had significant differences in the parasitism rate, with values reaching 33.28 and 37.2 respectively (Table 2). These treatments are the ones which have closer values to the positive control and hence they might have insecticide effect on *T. pretiosum* pupae.

At the third day, the extracts of *M. glomerata, V. curassavica, C. ambrosioides, V. polyanthes, P. amboinicus, A. absinthium* and *C. citratus* induced changes on the parasitism, resulting in significant differences when compared to the negative control and thus were classified as slightly harmful (class 2). However, the pupae treated with *A. absinthium* and *V. curassavica* had significant decrease in the parasitism and parasitism capability, denoting an increased effect of these treatments (Table 3). It is possible that at the moment of the emergence, residual treatment might have entered in contact with the insect through the shell opening, thus increasing the amount of extract ingested [20].
Table 1. Parasitism and viability on the 1st day after emergence (D.A.E.) in pupas of *Trichogramma pretiosum* (Hymenoptera: Trichogrammatidae)

<table>
<thead>
<tr>
<th>Treatments</th>
<th>1st D.A.E</th>
<th>% E</th>
<th>C</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>R. officinalis</em></td>
<td>63.08±0.75 cd</td>
<td>21.6</td>
<td>1</td>
</tr>
<tr>
<td><em>M. glomerata</em></td>
<td>65.92±6.17 bc</td>
<td>18.07</td>
<td>1</td>
</tr>
<tr>
<td><em>V. curassavica</em></td>
<td>75.27±4.81 ab</td>
<td>6.44</td>
<td>1</td>
</tr>
<tr>
<td><em>C. ambrosioides</em></td>
<td>65.62±3.53 bc</td>
<td>18.45</td>
<td>1</td>
</tr>
<tr>
<td><em>V. polyanthes</em></td>
<td>66.90±6.67 bc</td>
<td>16.85</td>
<td>1</td>
</tr>
<tr>
<td><em>P. amboinicus</em></td>
<td>63.77±3.05 cd</td>
<td>20.74</td>
<td>1</td>
</tr>
<tr>
<td><em>T. riparia</em></td>
<td>59.81±7.11 cd</td>
<td>25.67</td>
<td>1</td>
</tr>
<tr>
<td><em>A. absinthium</em></td>
<td>55.85±4.44 e</td>
<td>30.59</td>
<td>2</td>
</tr>
<tr>
<td><em>C. ambrosioides</em></td>
<td>64.12±3.74 cd</td>
<td>20.32</td>
<td>1</td>
</tr>
<tr>
<td>Destilled water</td>
<td>80.46±1.71 a</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Chlorpyrifos</td>
<td>12.93±0.99 f</td>
<td>83.94</td>
<td>3</td>
</tr>
</tbody>
</table>

Means ± SE with the same letter in the column do not differ by Turkey’s test (P>0.05); 1 Parasitism; 2 Reduction in viability capacity; 3 Classes: 1 - harmless (E < 30%), 2 - slightly harmful (30 ≤ E ≤ 79%), 3 - moderately harmful (80 ≤ E ≤ 99%) and 4 - harmful (E > 99%)

Table 2. Parasitism and viability on the 2nd day after emergence on pupas of *Trichogramma pretiosum* (Hymenoptera: Trichogrammatidae)

<table>
<thead>
<tr>
<th>Treatments</th>
<th>2nd D.A.E</th>
<th>% E</th>
<th>C</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>R. officinalis</em></td>
<td>57.52±0.58 b</td>
<td>15.83</td>
<td>1</td>
</tr>
<tr>
<td><em>M. glomerata</em></td>
<td>46.75±1.76 c</td>
<td>31.59</td>
<td>2</td>
</tr>
<tr>
<td><em>V. curassavica</em></td>
<td>57.51±2.36 b</td>
<td>15.85</td>
<td>1</td>
</tr>
<tr>
<td><em>C. ambrosioides</em></td>
<td>44.74±1.63 c</td>
<td>34.53</td>
<td>2</td>
</tr>
<tr>
<td><em>V. polyanthes</em></td>
<td>42.47±2.35 cd</td>
<td>37.85</td>
<td>2</td>
</tr>
<tr>
<td><em>P. amboinicus</em></td>
<td>46.40±1.87 c</td>
<td>32.12</td>
<td>2</td>
</tr>
<tr>
<td><em>T. riparia</em></td>
<td>59.70±4.37 b</td>
<td>12.65</td>
<td>1</td>
</tr>
<tr>
<td><em>A. absinthium</em></td>
<td>33.28±3.29 e</td>
<td>51.31</td>
<td>2</td>
</tr>
<tr>
<td><em>C. citratus</em></td>
<td>37.20±2.89 de</td>
<td>45.57</td>
<td>2</td>
</tr>
<tr>
<td>Destilled water</td>
<td>68.34±1.55 a</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Chlorpyrifos</td>
<td>15.31±1.52 f</td>
<td>77.6</td>
<td>2</td>
</tr>
</tbody>
</table>

Means ± SE with the same letter in the column do not differ by Turkey’s test (P>0.05); 1 Parasitism; 2 Reduction in viability capacity; 3 Classes: 1 - harmless (E < 30%), 2 - slightly harmful (30 ≤ E ≤ 79%), 3 - moderately harmful (80 ≤ E ≤ 99%) and 4 - harmful (E > 99%)

Table 3. Parasitism and viability on the 3rd day after emergence on pupas of *Trichogramma pretiosum* (Hymenoptera: Trichogrammatidae)

<table>
<thead>
<tr>
<th>Treatments</th>
<th>3rd D.A.E</th>
<th>% E</th>
<th>C</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>R. officinalis</em></td>
<td>34.92±0.43 b</td>
<td>13.03</td>
<td>1</td>
</tr>
<tr>
<td><em>M. glomerata</em></td>
<td>15.15±0.83 de</td>
<td>62.27</td>
<td>2</td>
</tr>
<tr>
<td><em>V. curassavica</em></td>
<td>10.98±0.77 f</td>
<td>72.88</td>
<td>2</td>
</tr>
<tr>
<td><em>C. ambrosioides</em></td>
<td>23.30±1.93 c</td>
<td>42.01</td>
<td>2</td>
</tr>
<tr>
<td><em>V. polyanthes</em></td>
<td>22.50±1.55 c</td>
<td>43.96</td>
<td>2</td>
</tr>
<tr>
<td><em>P. amboinicus</em></td>
<td>20.12±1.57 c</td>
<td>49.91</td>
<td>2</td>
</tr>
<tr>
<td><em>T. riparia</em></td>
<td>12.18±2.43 ef</td>
<td>69.68</td>
<td>2</td>
</tr>
<tr>
<td><em>A. absinthium</em></td>
<td>9.15±0.56 f</td>
<td>77.2</td>
<td>2</td>
</tr>
<tr>
<td><em>C. citratus</em></td>
<td>16.36±1.40 d</td>
<td>59.26</td>
<td>2</td>
</tr>
<tr>
<td>Destilled water</td>
<td>40.16±1.37 a</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Chlorpyrifos</td>
<td>2.48±0.23 g</td>
<td>93.82</td>
<td>3</td>
</tr>
</tbody>
</table>

Means ± SE with the same letter in the column do not differ by Turkey’s test (P>0.05); 1 Parasitism; 2 Reduction in viability capacity; 3 Classes: 1 - harmless (E < 30%), 2 - slightly harmful (30 ≤ E ≤ 79%), 3 - moderately harmful (80 ≤ E ≤ 99%) and 4 - harmful (E > 99%)
V. curassavica has anti-inflammatory effect, with several compounds in the aerial portion, being examples the tannins, flavonoids and essential oils [21]. Experiments made to assess the effect of macerated V. curassavica on larvae and pupae of S. frugiperda resulted in a longer duration of these juvenile stages, an effect that might be associated to the reduction of the food digestion efficiency [22]. Similarly, it is possible that these compounds are also related to the decrease of the parasitism capability in females of T. pretiosum. A. absinthium plants are commonly used in the popular medicine, having insecticide action and being rich in essential oils, terpenoids and absinthin [23].

In an experiment made with essential oil of A. absinthium applied over wheat seeds, it was observed repellent and insecticide effect against Tribolium castaneum (COLEOPTERA: TENEBRIONIDAE) in concentrations of 60 µL, with higher mortality rates being reached afterwards 48 hours [24]. It is likely that this repellent action might be the cause of the parasitism reduction in females of T. pretiosum.

There is a common misconception that botanical products have insecticide effect at the same time that they are safer and less toxic to the environment in relation to synthetic products. However, there are several botanical products in the market which are toxic to fishes, pollinators, mammals, beneficial insects, natural enemies, among other examples. Hence, the testing of botanical products becomes an important research step, aiming to verify their effects on pest control and selectiveness to natural enemies [25].

In this context, it might be said that among all the botanical extract tested in the present work, solely R. officinalis resulted in selectiveness to pupae of T. pretiosum. The remaining treatments caused some sort of negative effect over this natural enemy. This information highlights the need of broader studies with botanical extracts in the semi-field and field environment, aiming to test their toxicological potential and assess the impacts of their usage.

3.2 Sublethal Effect of Extracts on Pupae of T. pretiosum

Sublethal effects may affect the growth and development, actively interfering in the cell metabolism [26]. This way, females of T. pretiosum which emerged from eggs pulverized with water, R. officinalis, M. glomerata, V. curassavica, C. ambrosioides, V. polyanthes, P. amboinicus and A. absinthium, had a mean of 25 parasitized eggs, with this number decreasing across the parasitoid life-span (Fig. 1). This parasitism decrease occurs naturally in the parasitoid natural history when they are not affected by any formulated product [27],[28].

The accumulated parasitism percentage reached 80% around the 8th day with pulverizations of distilled water, R. officinalis, M. glomerata, V. curassavica, C. ambrosioides, P. amboinicus, T. riparia, A. absinthium and C. citratus. The positive control, chlorpyrifos, initially had a mean of four parasitized eggs, with 80% of accumulated parasitism being reached with 2.5 days (Fig. 1), confirming the toxic effect to Trichogramma [29].

When pupae which received treatments of M. glomerata, V. curassavica, C. ambrosioides, V. polyanthes, P. amboinicus, T. riparia, A. absinthium, C. citratus had their longevity and number of accumulated eggs in female adults evaluated, it was verified significant differences in different intensities when compared to the positive control. However, treatments of R. officinalis and C. citratus had more significant results, both being closer to the positive control in terms of parasitized eggs and female longevity (Table 4).

Tests with liquid extracts of R. officinalis (prepared with leaves immersed in methanol for 48 hours, evaporated with rotavapor, with 0.070 g solved in 8 ml of 1% tween 80 solution) on larvae of Leucoptera coffeella (LEPIDOPTERA: LYONETIIDAE), infesting coffee leaves resulted in 27% mortality, 48 hours afterwards a 10 minutes immersion into the extract, with this measure being attributed to the terpene present in this plant [30]. In larvae of Musca domestica (DIPTERA: MUSCIDAE), 1% citral caused a 100% mortality and demonstrated the larvicide effect of C. citratus [31].

The effects of the positive control, chlorpyrifos, on pupae of T. pretiosum is clear. Even with the parasitoid emergence, the number of parasitized eggs and female longevity have decreased (Table 4). Depending on which development stage the insect is, the application of insecticides does not interfere in the emergence [32].
In face of all results presented, it is possible to say that all botanical extracts in this experiment induced some sublethal effect on the tested pupae, with the extracts of *R. officinalis* and *C. citratus* causing higher damage than the others. Thus, it is important to highlight the need for studies evaluation the sublethal effects as a complement to the selectivity tests proposed by the IOBC. The present results will aid in the development of a promising IPM strategy using parasitoids and plant extracts.

**Table 4. Biological parameters on pupas of *Trichogramma pretiosum* (Hymenoptera: Trichogrammatidae) under effect of the treatments**

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Nº parasitized eggs</th>
<th>Longevity (D)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>R. officinalis</em></td>
<td>78,09 ± 1,02 e</td>
<td>4,5 ± 0,12 e</td>
</tr>
<tr>
<td><em>M. glomerata</em></td>
<td>98,6 ± 1,08 bc</td>
<td>8,4 ± 0,22 bc</td>
</tr>
<tr>
<td><em>V. curassavica</em></td>
<td>101,36 ± 0,63 b</td>
<td>7,45 ± 0,23 bcd</td>
</tr>
<tr>
<td><em>C. ambrosioides</em></td>
<td>101,18 ± 0,6 b</td>
<td>7,54 ± 0,12 bcd</td>
</tr>
<tr>
<td><em>V. polyanthes</em></td>
<td>95,9 ± 0,73 c</td>
<td>7,4 ± 0,12 cd</td>
</tr>
<tr>
<td><em>P. amboinicus</em></td>
<td>85,7 ± 0,75 d</td>
<td>7,3 ± 0,26 d</td>
</tr>
<tr>
<td><em>T. riparia</em></td>
<td>94,9 ± 0,73 c</td>
<td>8,5 ± 0,16 b</td>
</tr>
<tr>
<td><em>A. absinthium</em></td>
<td>82,2 ± 0,86 de</td>
<td>6,6 ± 0,12 d</td>
</tr>
<tr>
<td><em>C. citratus</em></td>
<td>60,5 ± 1,46 f</td>
<td>4,5 ± 0,12 e</td>
</tr>
<tr>
<td>Destilled water</td>
<td>107,09 ± 0,38 a</td>
<td>9,63 ± 0,21 a</td>
</tr>
<tr>
<td>Chlorpyrifos</td>
<td>0,53 ± 0,17 g</td>
<td>1,2 ± 0,09 f</td>
</tr>
<tr>
<td>CV</td>
<td>4,56</td>
<td>11,38</td>
</tr>
</tbody>
</table>

*Means ± SE with the same letter in the column do not differ by Turkey’s test (P>0,05)*

---

*Fig. 1. Daily and accumulated parasitism of *Trichogramma pretiosum* (Hymenoptera: Trichogrammatidae) under effect of the treatments*
4. CONCLUSION

We conclude that the tested botanical extracts are innocuous (class 1) and slightly harmful (class 2) to pupae of *T. pretiosum*, causing sublethal effects that might spoil the natural efficiency of the parasitoid when used in the Integrated Pest Control.

ACKNOWLEDGEMENTS

The T.R.T. was partial funded by the Brazilian Federal Agencies: “Coordenação de Aperfeiçoamento de Pessoal de Nível Superior” (CAPES), Brazil – Finance Code 001, and by the “Conselho Nacional de Desenvolvimento Científico e Tecnológico” (CNPq), Brazil. We would also like to thank the “Fundação de Amparo e Pesquisa do Estado de São Paulo” (FAPESP), for funding the research project no. 2013/22435, which this research is part of.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

13. Hassan SA. Guidelines for testing the effects of pesticides on beneficials organisms: description of test methods. In: Guidelines for testing the effects of


© 2020 Gladenucci et al.; This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sdiarticle4.com/review-history/62763