General Entomology

Selectivity and sublethal effect of botanical extracts on adults of *Trichogramma pretiosum* Riley (Hymenoptera: Trichogrammatidae)

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Abstract. The objective was to assess the selectivity and sublethal effect of botanical extracts on adults of *Trichogramma pretiosum* Riley (Hymenoptera: Trichogrammatidae). Plant species used: *Rosmarinus officinalis* L., *Mikania glomerata* Spreng., *Varronia curassavica* Jacq., *Chenopodium ambrosioides* L., *Vermonia polyanthes* Spreng., *Plectranthus amboinicus* Spreng., *Tetradenia riparia* Codd., *Artemisia absinthium* L., *Cymbopogon citratus* Stapf. Plant material were reared in laboratory and leaves collected during the morning were used to prepare the extracts as following: 100 g of leaves from each plant were macerated and immersed in 300 mL of triple-extracted methyl alcohol and evaporated on rotavapor. Then, 250 mg of crude extract was diluted in 1000 mL of water. The experimental design for selectivity was done in randomized blocks with five repetitions, following a standardized protocol of the International Organization for Biological Control (IOBC) and accordingly classified. In sublethal effect, the design was completely randomized with twenty repetitions, where the eggs exposed to parasitism were sprayed with the treatments. In both experiments the control was twofold (water and chlorpyrifos). Regarding selectivity, the botanical extracts of *M. glomerata*, *C. ambrosioides*, *V. polyanthes*, *P. amboinicus*, *A. absinthium* and *C. citratus* were classified as slightly harmful (class 2), chlorpyrifos as moderately harmful (class 3). In the evaluation of sublethal effects, the botanical extracts of *C. ambrosioides* and *C. citratus* initially presented reduced number of parasitized eggs, reduction in total parasitized eggs and female longevity. It can be concluded that *C. citratus* botanical extract has small selectivity and causes sublethal effects on *T. pretiosum* adults.

Keywords: Biological Control; Botanical insecticides; Entomology; Integrated Pest Management; Parasitoid.

The evaluation of selective products is done by using natural enemies of agricultural pests, with members of the parasitoid wasp genus *Trichogramma* being indicated as subjects by the International Organization for Biological Control (IOBC) standardized protocols. *Trichogramma* are parasite wasps of lepidopteran eggs species which are considered to be agricultural pests. The genus has cosmopolite distribution, with several species having their hosts associated, a trait that favors massive breeding and hence also favors their usage in biological control (Parra *et al*. 2015).

Previously, selectivity traits used to classify insecticides were based on short-term lethality parameters over natural enemies of pests. However, even though some of these insecticides may not quickly kill the subject, they may lead to sublethal effects such as: lowering of the longevity and ability to prey/parasitize, changing in different levels of biological or behavioral characters, lowering of the proper biological development, morphological deformations and lowering of reproductive traits (fecundity/fertility/fitness), among other problems (Croft 1990; Desneux *et al*. 2007; Vogt *et al*. 2007). Botanical extracts were largely used in agricultural and medical pest control as insecticides between 1930 and 1950, but were later substituted by synthetic molecules (Machado *et al*. 2007; Martinez 2011). From these compounds, several chemical substances synthetized in the secondary metabolism

of plants can be obtained and be used as insecticides, allowing the expansion of agroecological products (Silva *et al*. 2017). However, one of the limitations of botanical insecticides usage is the lack of knowledge about the effect of these compounds on the benefic fauna of agroecosystems (Vendramim & Castiglioni 2000), especially in regard to pest's natural enemies. The majority of works dealing with pest management by using botanical products highlight that the usage of these substances must be compatible with other pest control tactics, especially biological control. However, some researches have been demonstrating the existence of variations in the effects caused by botanical products on natural enemies (Ribeiro *et al*. 2009).

Several natural or botanical products are commonly related as causers of negative effects in *Trichogramma* spp. One of the most famous examples is the Neem (*Azadirachta indica* A. Juss), which is sprayed in host-eggs, resulting in significant reduction of the parasitism (Oliveira *et al*. 2003; Gonçalves-Gervásio & Vendramim 2004; Hohmann *et al*. 2010; Silva 2010). The Azidrachtin is the main compound among the secondary compounds. However, several other triterpenoids are found, such the meliantriol, limonene, oderatone, as well as more than 150 other compounds already isolated, which may present synergy with insecticides, enhancing its action (Sidiqui *et al*. 2003; Morgan 2009).

However, despite the benefits above-mentioned, these plants have the disadvantage of lacking data related to phytotoxicity, persistency and their effects in benefic organisms (Costa *et al*. 2004; Aguiar-Menezes 2005). Plant effects can be measured by checking if there is lowering in feeding, inhibition of chitin production, growth interruption, changings in ecdysis, reduction in fecundity, morphological deformations, increasing in death rate and repellency and toxicity to men and domestic animals (Medina *et al*. 2003; Mossini & Kemmelmeier 2005; Ndione *et al*. 2007; Morgan 2009).

One of the biggest challenges in the usage of plants as bioinsecticides is the elaboration of formulas which are able to keep their ingredients active through longer periods of time in the target body (Nerio *et al*. 2009), aside from getting information about biologic control agents and the effects of plant-based products.

Thus, it is demonstrated the need for development of new studies about the effect of natural products in agents of biological control, as new products are still arriving at the market. In this context, the aim of this work was to investigate the selectivity and sublethal effects of botanical extracts in adults of *Trichogramma pretiosum* Riley.

MATERIAL AND METHODS

The experiment was conducted at the Plant Protection Department of the Faculty of Agronomic Sciences, *Universidade Estadual Paulista "Júlio de Mesquita Filho"* (FCA/UNESP), Botucatu Campus, São Paulo, Brazil, under controlled conditions (12 h of photophase, 25 °C e 70% of relative humidity).

Plant collect and botanical extract preparation. The extracts were obtained at the Laboratory of Medicinal Plants of the Horticulture Department of the Faculty of Agronomic Sciences, *Universidade Estadual Paulista Júlio de Mesquita Filho"* (FCA/UNESP). Leaves were collected during morning, reared under organic management at Medicinal Garden and then taken to the laboratory. A hundred grams of fresh material of each plant were macerated in methanol (immersed in 300 mL of methyl alcohol for 24, 48 and 72 h, in triple extraction, resulting in 900 mL of methanolic extract). The solvent was evaporated in a rotatory evaporator (Quimis) and the crude extract diluted in the proportion of 1000 mL to 250 mg of distilled water, then conserved in a glass recipient of amber coloration.

Rearing maintenance of *T. pretiosum*. Adults of *T. pretiosum* were kept in a four liters recipient, under controlled conditions of temperature (25 ± 2 °C), relative humidity (70 ± 10%) and photophase (14 h), fed with crude honey chunks; fresh eggs of *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae), glued to a double-sided tape which was glued over a coated paper, were available during 24 h to stimulate parasitism. Past this time period, the cards were individualized in new recipients until the larvae emerged from the eggs, starting a new rearing cycle.

Treatment selection. The botanical extracts of *Rosmarinus officinalis* L., *Mikania glomerata* Spreng., *Varronia curassavica* Jacq., *Chenopodium ambrosioides* L., *Vermonia polyanthes* Spreng., *Plectranthus amboinicus* Spreng., *Tetradenia riparia* Codd., *Artemisia absinthium* L., *Cymbopogon citratus* Stapf used in the bioassays were chosen based on previous results obtained by testing in second instar nymphs of *Spodoptera frugiperda* (Smith) (Lepidoptera: Noctidae). The tests consisted of pulverizing the extracts on the nymphs by using a Potter spray tower® (by contact) and by incorporating the extracts in the artificial diet (by ingestion). Thus, the criteria of the plant species selection were the insecticide effect of

the plants, by contact and ingestion, in the above-mentioned pest insect, in the concentration of 1000 mL of crude extract to 250 mL of distilled water, as previously described. The active ingredient chlorpyrifos, an insecticide of the organophosphate chemical group, was used as positive control (as recommended by IOBC) and distilled water as negative control.

Application of botanical extracts in selectivity and sublethal bioassays. The experimental design was made in randomized blocks with eleven treatments and five repetitions for selectivity and entirely randomized with 20 repetitions for sublethal effect. The Potter spray tower® was pre-calibrated to pulverize 250 L ha^{-1}, with pressure varying between 5-7 psi and deposition of 1.75 ± 0.25 mg cm^2, measured in analytical balance, following the standard procedure suggested by the IOBC. For the pulverization, a volume of 1.13 mL of each treatment was separated in Eppendorfs®.

Cages for selectivity assessment. The cages used followed the IOBC suggestion, made with aluminum frames juxtaposed with screws. Three sides of the cage have entirely tightened orifices, assuring the imprisonment of the subject and allowing for gas exchange (Hassan *et al*. 2000). On the fourth side there are two openings: one made to introduce the egg-cards and another used to release the parasitoids in the cages. The top and bottom sides are sealed with glass plates and interconnected by a hose-system connected to a pressure pump used to remove air by suction.

Selectivity. The top and bottom glass-plates of the cages were built to expose the subjects to the pulverizations. Prior to the exposure, cards with parasitized eggs (± 600 eggs) were individualized in Duran® tubes to obtain a mean of 200 females per cages (Hassan *et al*. 2000). The individuals of 24 hours-old were exposed to the treatments. After the colonization of the interior of the cages, cards of 6.25 cm² with fresh eggs of *E. kuehniella* (± 1.200 eggs) were made available to stimulate parasitism, during the period of 24, 48 and 72 h. After 96 hours, the cages were disassembled and the egg-cards collected and individualized by date and treatment in plastic bags to posterior analyses. The parasitism (P) was analyzed with the equation P = number of parasitized eggs / number of offered eggs x 100. The viability and parasitism reduction in relation to the negative control was assessed by the efficiency equation: E (%) = (1 – (mean percentage of the treatment / mean percentage of the treatment control) x 100, where: E (%) is the percentage reduction of the benefic capacity of the parasitoid (Sterk *et al*. 1999; Manzoni *et al*. 2006). Thus, the results were classified according to mean reduction, as suggested by the IOBC standards: class 1 - innocuous (E < 30%); class 2 – slightly harmful (30 ≤ E ≤ 79%); class 3 – moderately harmful (80 ≤ E ≤ 99%); class 4 – harmful (E > 99%) (Hassan 1992).

Sublethal effects. Females of *T. pretiosum* were individualized in a Duran® tube based on antenna structure (Querino & Zucchi 2011) and fed with a drop of honey. During their life-time, the females were daily offered cards of 0.25 cm² with fresh eggs (± 180 eggs) of *E. kuehniella*, pulverized with the treatments, to observe the sublethal effects of those females which did not suffer lethal effect. The egg-cards were labeled and stored to posterior analyses. The total number of parasitized eggs and longevity of parasitoids was analyzed, although the sublethal effects of the treatments may affect the insect's physiology and interfere in the analyzed parameters (Desneux *et al*. 2007).

Statistical analyses. The results were analyzed by an Analyses of variance (ANOVA) and the means compared with a Tukey's test with 5% of significance with the software SISVAR 5.6.

RESULTS AND DISCUSSION

Selectivity of botanical extracts on adults of *T. pretiosum*. The usage of selective products, be chemicals or botanical extracts, contribute to the efficiency of the natural and applied biological control, being less aggressive to the benefic entomofauna. However, the botanical active ingredients are composed by a complex of substances, which can have insecticide effects and thus may toxically act in the insect's body, leading to feeding inhibition, difficulty in growth, development, reproduction and changing in behavior (Aguiar-Menezes 2005). Hence, selective insecticides are more suitable to be used in pest control with less impact in the populations of natural enemies (Bueno & Bueno 2012).

The highest parasitism rates occurred on the first day afterwards emergence (D.A.E.), when adults of *T. pretiosum* entered in contact with pulverized surfaces. However, the extracts of *M. glomerata*, *C. ambrosioides*, *V. polyanthes*, *P. amboinicus*, *A. absinthium* and *C. citratus* caused alterations of the parasitism, being classified as slightly harmful (class 2). The extract of *C. citratus* caused alteration of the parasitism (16.18), as well as reduction (76.44%), numbers close to those of the positive control (chlorpyrifos), which is considered to be moderately harmful (class 3) (Table 1).

On the second D.A.E., the extracts of *R. officinalis*, *M. glomerata*, *C. ambrosioides* and *V. polyanthes* induced the reduction of the parasitism and were classified as slightly harmful (class 2), as well as the extract of *C. citratus*, with a parasitism proportion of 6.74 and reduction of 78.99%, numbers which are also close to those of chlorpyrifos, with 1.83 and 94.3% respectively (Table 2).

On the third D.A.E., the extracts of *M. glomerata*, *C. ambrosioides*, *V. polyanthes*, *C. citratus* and chlorpyrifos were classified as slightly harmful (class 2) (Table 3). The remaining treatments did not cause changings or reductions of the parasitism.

Plants of *C. citratus* have therapeutic properties, such as bactericide and fungicide, and are also popularly used against lices (Lorenzi & Matos 2008). These features are possible due to the presence of terpenes, like the citral (a mix of neral and geranial isomers), myrcene, citronellol and nerol (Carriconde *et al*. 1996). Tests with three different concentrations (1%, 5% and 10%) of lemon grass essential oil in *Thyrinteina arnobia* Stoll (Lepidoptera: Geometridae) resulted in insecticide effect, causing 100% of mortality in subjects submitted to concentrations of 5% and 10% (Soares *et al*. 2011). This lethal effect in *T. arnobia* may explain the low amount of selectivity found in *T. pretiosum*. The chlorpyrifos is commonly included

Table 1. Parasitism and viability on the 1st day after emergence (D.A.E.) in adults of *Trichogramma pretiosum* (Hymenoptera: Trichogrammatidae).

Treatments	1st D.A.E			1st D.A.E		
	P[1]	% E[3]	C[4]	V[2]	% E[3]	C[4]
R. officinalis	54.64±0.71 b	20.43	1	95.22±0.51 ab	4.47	1
M. glomerata	36.85±1.59 d	46.34	2	96.73±1.42 ab	2.96	1
V. curassavica	52.20±1.94 b	23.98	1	78.34±5.58 c	21.41	1
C. ambrosioides	36.73±2.51 d	46.52	2	99.09±0.05 a	0.11	1
V. polyanthes	42.70±2.80 c	37.52	2	95.05±1.15 ab	4.65	1
P. amboinicus	44.60±2.43 c	35.07	2	97.61±0.35 a	2.08	1
T. riparia	51.12±4.89 b	25.57	1	95.53±1.99 ab	4.16	1
A. absinthium	43.76±2.90 c	36.27	2	90.63±3.77 b	9.07	1
C. citratus	16.18±0.59 e	76.44	2	93.15±3.10 ab	6.55	1
Distilled water	68.67±0.93 a	0	1	99.67±0.09 a	0	1
chlorpyrifos	6.23±0.85 f	90.92	3	79.01±6.56 c	20.74	1

Means ± SE with the same letter in the column do not differ by Tukey's test (P>0,05). [1]Parasitism; [2]viability; [3]Reduction in viability capacity; [4] Classes: 1 - innocuous (E < 30%), 2 – slightly harmful (30 ≤ E ≤ 79%), 3 – moderately harmful (80 ≤ E ≤ 99%) and 4 - harmful (E > 99%).

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

Treatments	2nd D.A.E[1]			2nd D.A.E[1]		
	P[2]	% E[3]	C[4]	V[2]	% E[3]	C[4]
R. officinalis	19.91±0.43 d	37.92	2	92.96±0.58 a	6	1
M. glomerata	14.17±0.86 e	55.82	2	94.07±1.42 a	4.88	1
V. curassavica	25.74±2.11 b	19.74	1	93.19±6.19 a	5.77	1
C. ambrosioides	16.30±1.36 e	49.19	2	97.67±1.53 a	1.31	1
V. polyanthes	16.33±1.09 e	49.07	2	94.55±1.65 a	4.4	1
P. amboinicus	22.89±1.25 bcd	28.65	1	87.78±3.74 ab	11.26	1
T. riparia	23.54±2.49 bc	26.61	1	97.12±0.45 a	1.79	1
A. absinthium	22.90±1.40 cd	28.6	1	95.84±2.05 a	3.09	1
C. citratus	6.74±0.90 f	78.99	2	91.60±6.59 ab	7.39	1
Distilled water	32.07±2.44 a	0	1	96.31±3.21 a	0	1
chlorpyrifos	1.83±0.16 g	94.3	3	81.34±11.72 b	23.1	1

Means ± EP followed by the same letter in the column do not differ between themselves in Tukey's test (P>0,05). [1]Parasitism; [2]viability; [3]Reduction in viability capacity; [4]Classes: 1 - innocuous (E < 30%), 2 – slightly harmful (30 ≤ E ≤ 79%), 3 – moderately harmful (80 ≤ E ≤ 99%) and 4 - harmful (E > 99%).

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

Treatments	3rd D.A.E			3rd D.A.E		
	P[1]	% E[3]	C[4]	V[2]	% E[3]	C[4]
R. officinalis	7.98±0.20 cd	25.43	1	87.69±0.41 cd	10.53	1
M. glomerata	6.20±0.45 e	42.1	2	94.83±2.43 ab	3.26	1
V. curassavica	8.50±0.16 c	20.5	1	84.31±5.85 d	13.98	1
C. ambrosioides	5.30±0.27 f	50.51	2	90.69±2.86 bcd	7.08	1
V. polyanthes	6.72±0.74 e	37.22	2	91.42±2.10 abc	6.74	1
P. amboinicus	7.70±0.33 d	28.1	1	87.82±4.61 cd	10.41	1
T. riparia	9.44±0.14 b	11.82	1	96.21±1.37 ab	1.84	1
A. absinthium	9.55±0.09 b	10.74	1	97.60±1.04 a	0.43	1
C. citratus	4.87±0.46 f	54.54	2	86.91±5.34 cd	11.33	1
Distilled water	10.79±0.22 a	0	1	98.02±0.56 a	0	1
chlorpyrifos	3.10±0.10 g	71.04	2	92.26±1.19 abc	5.88	1

Means ± SE with the same letter in the column do not differ by Tukey's test (P>0,05). [1]Parasitism; [2]viability; [3]Reduction in viability capacity; [4] Classes: 1 - innocuous (E < 30%), 2 – slightly harmful (30 ≤ E ≤ 79%), 3 – moderately harmful (80 ≤ E ≤ 99%) and 4 - harmful (E > 99%).

in tests of selectivity as positive control of toxicity to allow for comparisons with other insecticides, due to its negative effects in *Trichogramma* species (Stefanello Júnior *et al*. 2008; Amaro *et al*. 2015).

Sublethal effects of botanical extracts on adults of *T. pretiosum*. Sublethal effects may affect growth and development, by interfering in cellular metabolism (Aguiar-Menezes 2005). The reduction of egg-laying rates or inhibition are important effects of botanical extracts over insect's reproduction (Costa *et al*. 2004). Analyzing the total number of parasitized eggs, in the treatments of chlorpyrifos, *C. ambrosioides* and *C. citratus*, the number of parasitized eggs was 0; 2.66 and 2.08 respectively (Table 4). In treatment with chlorpyrifos, it is possible that the high toxicity of the organophosphate compounds may have interfered in the results. Souza (2011) reported a mortality of half of the population of *T. pretiosum* after one hour of exposition to eggs treated with chlorpyrifos.

In the *C. ambrosioides* treatment, it may have happened due to the presence of ascaridole, a secondary compound. Experiments made with essential oil made from leaves and stem of *C. ambrosioides*, in the weevil *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae), resulted in a 100% mortality rate, 24 h after exposition to a dose of 500 μL oil/L (Jaramillo *et al*. 2012). The treatment *C. citratus*, which has citral among its secondary compounds, may have affected the amount of parasitized eggs. Experiments made with essential oils of *Pelargonium graveolens* and *Lippia alba*, which possess geraniol, linalool, carvone and citral among their major compounds, resulted in significant mortality in larvae of *S. frugiperda* in comparison with the natural bioinsecticide Azamax® and with the negative control (Niculau *et al*. 2013).

In agroecosystems, the longevity of egg-parasitoids may be influenced by several factors, such as feeding, temperature, relative humidity, as well as nutritional conditions of the host-egg (Pacheco & Corrêa-Ferreira 1998).

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

Treatments	Nº parasitized eggs		Longevity (D)	
R. officinalis	44.6±2.34	f	3.2±0.39	bcd
M. glomerata	107.28±7.29	bc	5.28±0.5	bc
V. curassavica	95.5±6.01	cd	6.16±0.57	b
C. ambrosioides	2.08±1.78	g	3.92±0.49	bcd
V. polyanthes	80.71±4.83	de	3.14±0.3	cde
P. amboinicus	65.44±6.97	e	4.33±0.54	bcd
T. riparia	80.88±3.95	de	3.88±0.38	bcd
A. absinthium	119.9±5.37	bc	4.8±0.38	bcd
C. citratus	2.66±1.39	g	2.6±0.24	def
Distilled water	165.57±6.82	a	10.42±0.57	a
chlorpyrifos	0	g	1.05±0.05	f

Means ± SE with the same letter in the column do not differ by Tukey's test (P>0,05).

In the present work, the parasitoid was exposed to botanical extracts in controlled laboratory conditions and it was observed alterations in female longevity, with the treatment *C. citratus* (2.6 days) being the closest one to the positive control chlorpyrifos (1.05 days). In treatment with distilled water, the duration was 10.42 days (Table 4), similar results to those of Oliveira *et al* (2005), in which the longevity had a variation between 11 and 13 days in laboratory conditions.

The effects of botanical extracts over the parasitoid's longevity may be related to the act of parasitize. When the female parasitoid starts touching the eggs pulverized with treatments, then she starts absorbing the toxic substances (by contact) and thus causing negative effects on the longevity. For a substance to cause toxic effects in an organism biology, it has to penetrate the cells and spread, starting to interfere in physiological or biochemical processes, causing impact in longevity, growth, development, reproduction and behavior (Delpuech & Meyet 2003).

The satisfactory parasitism rate in laboratory rearing is around 80%. In the present experiment, the distilled water reached 80% of parasitism by the 10th day, as well as the botanical extracts of *C. ambrosioides* and *V. polyanthes*. The treatments with *A. absinthium*. *R. oficinais*, *V. curassavica* and *C. citratus* reached the 80% rate sooner than expected, when comparing with distilled water. There was an extrapolation with the extract of *M. glomerate*, which reached 80% of parasitism only by the 15th day, contrary of *P. ambrosioides*, which reached the 80% rate by the sixth day, both treatments differing from the remaining (Figure 1).

Regarding the daily parasitism rate, the treatment with *C. citratus* differed from all the others, with approximately 15 parasitized eggs in the beginning, with several peaks of oviposition and days when there was no parasitism. The remaining treatments began with a mean of 25 parasitized eggs, then gradually diminishing over time. It was not possible to calculate the daily and accumulated parasitism rates of the positive control, chlorpyrifos, as all females died in the first 24 h (Figure 1).

According to MOREIRA *et al*. (2005), there is a misconception that botanical extracts with insecticide activity have a smaller toxic potential and are safer than the synthetical products, when there are botanical products in the market that are toxic to fishes, pollinators, mammals, benefic insects, natural enemies, etc. In this context, researches to find botanical products that are efficient in controlling the pest, but selective to natural enemies, are especially important.

Given the results found, we conclude that the botanical extracts analyzed are innocuous (class 1) or slightly harmful (class 2) to *T. pretiosum*. However, it is demonstrated that *C. citratus* has small selectivity and sublethal effect on adults of *T. pretiosum*.

The treatments classified as class 1 may be recommended to integrated pest management programs, aiming to unite alternative and biological methods. The remaining treatments need more studies about their selectivity, which should be done in semi-field and field conditions, to confirm their toxic potential and investigate their impacts.
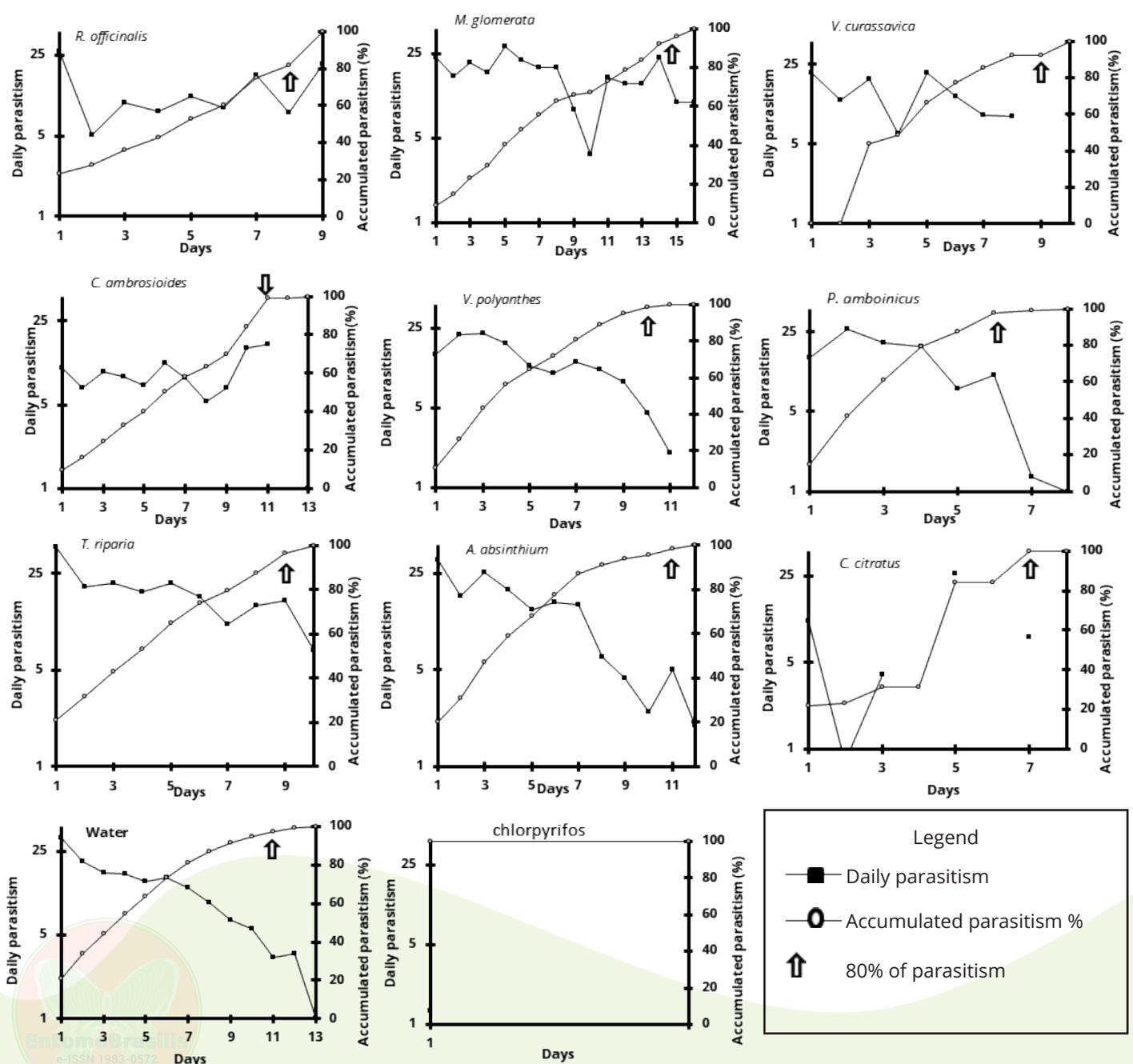
Figure 1. Daily and accumulated parasitism of *Trichogramma pretiosum* (Hymenoptera: Trichogrammatidae) under effect of the treatments.

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