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Resumo

Ensaios de laboratório e de campo têm demonstrado que os parasitóides do género *Trichogramma* são muito suscetíveis à maioria dos inseticidas de largo espectro, reduzindo a sua eficácia como agentes de controlo biológico. A integração deste agente de controlo biológico com outros métodos requer o conhecimento dos efeitos letais e subletais que os insecticidas podem ter sobre estes inimigos naturais. De modo a promover métodos ambientalmente seguros de controlo de pragas nos Açores, este trabalho teve como objetivo avaliar a segurança de óleos essenciais de *Pittosporum*m *undulatum* e *Hedychium gardnerianum* em diversos parâmetros biológicos do parasitóide oófago *Trichogramma cordubensis*, quando aplicado sobre ovos do hospedeiro, antes e depois do parasitismo. Para tal, avaliou-se os efeitos letais e sub-letais destes óleos às concentrações de 0.5% e 2.5%, por contacto direto e residual, na longevidade e fecundidade de fêmeas *T. cordubensis*, e na percentagem de emergência e tempo de desenvolvimento preimaginal da descendência. O óleo essencial que mais afetou os parâmetros biológicos do parasitóide, tanto por contato residual ou direto, foi *H. gardnerianum* à concentração de 2.5%. O número de ovos parasitados e a longevidade de *T. cordubensis* foram os parâmetros mais negativamente afetados. *H. gardnerianum* à concentração de 2.5%, teve um efeito residual elevado enquanto que nos outros tratamentos verificou-se o enfraquecimento do efeito ao longo do tempo. Quando aplicado sobre os ovos parasitados, as fases de desenvolvimento embrionário e larval foram as mais afectadas por *H. gardnerianum* a 2.5%. Os óleos essenciais de *P. undulatum*, às concentrações de 2.5% e 0.5%, e *H. gardnerianum* à concentração de 0.5% são compatíveis com libertações de *T. cordubensis*, visto que a estas concentrações têm um efeito residual reduzido e, quando aplicados após a fase larvar do parasitóide, os seus efeitos são neglígiveis. *P. undulatum*, à concentração de 0.5%, apresentou resultados promissores, indicando que pode ser usado com segurança em qualquer altura de exposição e fase de desenvolvimento de *T. cordubensis*. No entanto, mais estudos devem ser realizados de modo a determinar o efeito destes óleos essenciais no comportamento biológico de *T. cordubensis*, em condições de campo.

Palavras-chave: *Trichogramma*, efeitos secundários, contato direto e residual, extratos de plantas, *Pittosporum undulatum*, *Hedychium gardnerianum.*

Abstract

Laboratory and field studies have shown that *Trichogramma* wasps are highly susceptible to most broadspectrum insecticides, reducing their efficacy as biological control agents. Thus, the integration of this biological control agent with other methods requires knowledge of the lethal and sublethal effects they may have on these natural enemies. To promote environmentally friendly pest control methods in Azorean crops, this research aimed at assessing the safety of essential oils of *Pittosporum undulatum* and *Hedychium gardnerianum* on several biological parameters of the wasp *Trichogramma cordubensis*, when applied on host eggs before and after parasitism. We evaluated the lethal and sublethal effects of these essential oils at the concentrations of 0.5% and 2.5%, by residual and direct contact, on the longevity and fecundity of *T. cordubensis* and, on the offspring emergence rate and preimaginal development time. The essential oil that most affected the biological parameters of *T. cordubensis*, either by residual or direct contact, was *H. gardnerianum* at the concentration of 2.5%. The number of parasitized eggs and the longevity of *T. cordubensis* were the parameters most negatively affected. At the concentration of 2.5%, *H. gardnerianum* showed to have a high residual effect while, for the other treatments we observed a reduction of this effect over time. When applied on parasitized host eggs, the embryo and larval developmental stages of *T. cordubensis* were the most negatively affected by the treatment with *H. gardnerianum* at 2.5%. Our results suggest that the essential oils from *H. gardnerianum* at the concentration of 0.5% and, from *P. undulatum* at the concentrations of 0.5% and 2.5%, are compatible with *T. cordubensis* releases, since at such concentrations these extracts have a low residual effect and, when applied after completion of the larval developmental stage, their side effects are negligible. Furthermore, *P. undulatum* at the concentration of 0.5% showed promising results, indicating that it can be safely used at any time of exposure and preimaginal developmental stage of the *T. cordubensis.* However, more attention should be devoted to field experiments to more clearly determine the influence of these essential oils to *T. cordubensis* under agricultural conditions.

Keywords: *Trichogramma*, side-effects, residual and direct contact, plant extracts, *Pittosporum undulatum*, *Hedychium gardnerianum*

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Chapter 1.

General Overview

The search for biologically active compounds from natural resources has always been of great interest to scientists looking for new sources and models for development of ecologically and environmentally friendly insect control agents (Isman, 2000; Lahlou, 2004; Isman *et al*., 2007; Rosa *et al*., 2009). The extensive use of pesticides is known to cause several problems in the environment, such as target pest resurgence and secondary pest outbreaks throughout the world's agroecosystems (Luck *et al*., 1977; Metcalf, 1986). Moreover, the reduction of the efficiency of biological control agents (BCA), such as parasitoids, in pesticide treated crop systems has been highlighted by several authors (Brunner *et al*., 2001; Hewa-Kapuge *et al*., 2003). Since insecticides may cause the death of the BCA (lethal effects) or change several other traits of their biology (either physiological or behavioural) without killing the individuals (sublethal effects), the success of Integrated Pest Management (IPM) programs depends, in part, on the optimal use of selective insecticides that are less harmful to natural enemies. Thus, in addition to direct mortality induced by insecticides, we should also have knowledge of the effects on physiological and behavioural traits of the natural enemies for a complete analysis of their impacts. (Desneux *et al*., 2007; Stark *et al*., 2007; Garcia, 2011).

An important way of avoiding this problem is the use of pesticides whose action spares natural enemies through either physiological or ecological selectivity (Saber *et al*., 2005). Essential oils, found in a variety of plants, have been suggested as an alternative source for insect control. These are volatile, natural, complex compounds characterized by a string odour, formed by aromatic plants belonging to a number of botanical families. Plants, as long-lived stationary organisms, must resist attackers over their lifetime, so they produce and exude constituents of the secondary metabolism, that play an important role in their defence mechanisms (Ntalli *et al*., 2011). Therefore, these chemical volatiles have functions in chemical defence, acting as insecticides (Bakkali *et al*., 2008), acaricides (Flamini, 2003), avoiding bacterial or fungi phytopathogen colonization (Karamanoli, 2002; Karamanoli *et al*., 2005), and/or attracting natural enemies of herbivores (Ntalli *et al*., 2011). Usually they are obtained by hydro-distillation and they comprise monoterpenes, sequiterpene lactones and triterpenes, which are examples of volatile plant compounds that may have commercial applications (Barney *et al*., 2005; Al-mazra'awi *et al*., 2009). Many of them are selective, biodegrade do nontoxic products and have few effects on the environment and non-target organisms (Isman, 2000). They can also provide an alternative for resistance

management because some plant extracts can be highly effective against insecticide-resistant insect pests (Isman, 2006; Ntalli *et al*., 2011). Thus, these plant compounds usually can be safely used in IPM (Schmutterer, 1992; Ahn *et al*., 1997; Yi *et al*., 2007).

The use of egg parasitoids for the control of pests has long been an essential part of pest management strategies in crop protection. Numerous egg parasitoids are effective natural enemies of important agricultural and forestry pests, killing the host before it causes any damage to the crops (Bastos *et al*., 2006). Among these, the genus *Trichogramma* Westwood has been considered for the control of pests for over than 100 years (Li-Ying, 1994). The genus *Trichogramma* is worldwide distributed and comprises about 145 described species. These minute wasps (0.2-1.5 mm) are solitary or gregarious endoparasitoids that can be easily produced in large scale using factitious hosts (Garcia, 1995). Moreover, the exploitation of their developmental pauses (diapause or quiescence) allows their storage at low temperatures for long periods, reducing the costs of mass rearing these insects (Garcia *et al*., 2002; Garcia *et al*., 2009). These egg parasitoids have a preference for Lepidoptera, but there are also records of parasitism on eggs of Coleoptera, Diptera, Hemiptera, Homoptera, Hymenoptera (Symphyta) and Neuroptera (Pinto *et al*., 1994).

Trichogramma cordubensis Vargas and Cabello (Hymenoptera: Trichogrammatidae) (Figure 1), a native thelytokous species of São Miguel Island (Azores, Portugal) is one of the most important species of egg parasitoids found on the island (Garcia *et al.*, 2006). According to Garcia (2000), this species is well adapted to the mild temperatures and high relative humidity that characterize the climate of the Azores, as well as to its natural hosts, that belong mostly to the family Noctuidae.

Figure 1. Female adult of *Trichogramma cordubensis* Vargas & Cabello (Hymenoptera: Trichogrammatidae), (30x).

Many studies have compared the relative toxicity of pesticides, including insecticides, fungicides and herbicides, to *Trichogramma* in screening trials. Laboratory and field studies have shown that these wasps are highly susceptible to most broad-spectrum insecticides, reducing their efficacy as BCA, by adversely affecting emergence rates (Hohmann, 1991, 1993; Suh *et al*., 2000; Takada *et al*., 2001; Vieira *et al*., 2001), adult mortality (Jacobs *et al*., 1984; Suh *et al*., 2000; Brunner *et al*., 2001; Vieira *et al*., 2001; Hewa-Kapuge *et al*., 2003) and parasitism of host eggs (Jacobs *et al*., 1984; Hagley *et al*., 1989; Hohmann, 1993; Hewa-Kapuge *et al*., 2003; Garcia *et al*., 2006). Thus, the integration of *Trichogramma* with chemical methods for pest management requires knowledge of the lethal and sublethal effects that the chemicals may have on the natural enemies, taking into particular consideration the developmental stages of the organism which will have the highest incidence of exposure (Yi *et al*., 2007).

To promote environmentally friendly pest control methods in Azorean crops, this research aimed at assessing the safety of the essential oils of *Pittosporum undulatum* Vent. (Pittosporaceae) and *Hedychium gardnerianum* Sheppard ex Ker-Gawler (Zingiberaceae), two invasive plant species of the Azores, to *T. cordubensis.* *Hedychium* is commonly known as ginger lilies and is a genus of herbs with thick, fleshy and branched rhizomes. Native to South Asia, the genus *Hedychium* has over 80 species, widely cultivated for ornamental purposes especially for their sweet scented flowers (Sabulal *et al*., 2007). *Heychium gardnerianum* was introduced in the Azores in the middle of the 19th century, spreading rapidly over the island of São Miguel, wherever the native forest becomes degraded, as well as being scattered in the dense laurel forest (Medeiros *et al*., 2003). The genus *Pittosporum* comprises about 150 species of tropical and subtropical Africa, Asia, Australia, New Zealand, and some Pacific Islands (Wagner *et al*., 1999). *Pittosporum undulatum* is an evergreen, 4-13m tree, with a fast growing leaf canopy, that is often used as an ornamental plant, due to its attractive fragrant flowers (Medeiros *et al*., 2003). It is native to south eastern Australia but has now spread to a great number of islands including the Azores. Its spontaneous spreading, has significantly transformed the islands' landscape, invading moist disturbed forests from low to middle elevations and threatening the survival of the native forest (Binggeli, 1998).

According to Rosa *et al*. (2009), the essential oils of these two plants are toxic and have ovicidal, growth inhibition and antifeedant activity against *Pseudaletia*

unipuncta (Lepidoptera: Noctuidae). This species, known as the armyworm, is a polyphagous insect and one of the most important pests of graminaceous crops, including pasture, in the Azores islands (Vieira *et al*., 2003). Other studies demonstrated that limolene and α-pinene, the main components present in the essential oils of these two plants, have been tested successfully against Coleopteran (Bekele *et al*., 2001; Ngamo *et al*., 2007) and other Lepidopteran pests (Miyazawa *et al*., 1998; Hummelbrunner *et al*., 2001). Furthermore, other recent studies have shown that the essential oils of *H. gardnerianum* display good molluscicidal (Teixeira *et al*., 2011; 2012) and antimicrobial activity against several *Staphylococcus* strains (Medeiros *et al*., 2003). Jadhav *et al*. (2007), reported *in vitro* insecticidal activity against human head lice by the essential oil of *Hedychium spicatum*, which is a species closely related to *H. gardnerianum*.

Therefore, and as suggested by Rosa *et al*. (2009), the essential oils of *H. gardnerianum* and *P. undulatum* can be exploited for the development of bioactive compounds as a new source of agrochemicals. Thus, in the present study we will evaluate the effects of these essential oils, by residual and direct contact, tested at two different concentrations, on the longevity and fecundity of the wasp *T. cordubensis* and, on the offspring emergence rates and preimaginal development times, to assess their possible integration in insect pest management programs.

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