



Effect of *Aronia melanocarpa* (Michaux) Elliott Leaf Extract on Different Life Stages of *Trichogramma evanescens* (Westwood, 1833) (Hymenoptera: Trichogrammatidae)

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Abstract

Demonstrating the effectiveness of botanical extracts and essential oils on insect pests has become one of the alternatives to control methods. In this respect, botanical pesticides are a curicial resource but need to have minimal impact on natural enemies. The objective of this study is to assess the impact of *Aronia melanocarpa* leaf extract on the biological characteristics of different life stages of *Trichogramma evanescens*. Parasitized *Ephestia kuehniella* egg cards were immersed in *A. melanocarpa* leaf extract solutions (0.1%, 0.5% and 1%) and dH₂O (control) for 5 seconds at 24 hours, 48 hours, 72 hours and 192 hours after parasitism, corresponding to the egg, larvae, prepupal and pupal stages of *T. evanescens*, respectively. The parasitization, emergence and sex ratio of *T. evanescens* were evaluated. The prepupal stage of parasitoid was more susceptible than other stages. Egg and pupal stages were more resistant than larvae and prepupal stages (Egg LC₅₀: 0,772%, Pupa LC₅₀: 0,412% respectively). This is the first study on the insecticidal effects of *A. melanocarpa* leaf extract on egg parasitoid *T. evanescens*. The leaf extract of *A. melanocarpa* completely at prevented adult emergence at a dose of 1% in the prepupal stage.

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1. Introduction

Egg parasitoids, particularly those of the genus *Trichogramma* (Hymenoptera: Trichogrammatidae), have been used for biological control programs far more frequently than other natural enemies. *Trichogramma* species prevents the hatching of the host insect from the egg to the larval stage and prevents it from damaging the plant (Wang et al., 2014). In this respect, its effectiveness in biological control is indisputable. In addition, its wide geographical distribution and simple rearing in the laboratory have made these natural enemies preferred in biological control programs. In Türkiye, the most common species is *T. evanescens* and has been detected in *Ostrinia nubilalis* eggs in the Black Sea, Marmara, and Mediterranean regions (Öztemiz, 2007).

Moreover, considering the importance of these natural enemies in biological control, they may still be insufficient against the target pest and may need to be used with alternative methods (Khan et al., 2015). Among these alternative control methods, chemical control should be the last resort to be considered within the scope of Integrated Pest Management (IPM) due to its negative effects on natural enemies. This situation has led researchers to discover environmentally friendly methods that are compatible with natural enemies in the control of insect pests, apart from chemical pesticides.

Investigation of aromatic plants and their powders, extracts and essential oils as biopesticides in terms of their insecticidal properties seems to be a good alternative (Lee et al., 2004). Many studies have been conducted on the effectiveness of botanical pesticides against stored product pests (Ercan et al., 2013). It is obvious that plants have a high potential in this sense and are an important resource. Unfortunately, some botanical pesticides can also be toxic to natural enemies (Regnault-Roger et al., 2012).

Aronia melanocarpa is a plant belonging to the Rosaceae family and is cultivated for fruit production. It grows naturally in the eastern region of North America and Canada. There

are two species of the plant known as "chokeberry", *A. melanocarpa* (black chokeberry) and *A. arbutifolia* (red chokeberry) (Kulling and Rawel, 2008). Due to its high antioxidant activity, it is used in the prevention and treatment of many diseases, and therefore its production and use are widespread throughout the world (Oszmiański and Lachowicz, 2016). There are many studies on the anticancer, antimutagenic and antibacterial activity of *Aronia* (Jurikova et al., 2017).

Many recent studies show that essential oils and plant extracts obtained from aromatic plants are successfully used in the control of stored product pests (Erler, 2005; Negahban et al., 2007; Ayvaz et al., 2009). Additionally, it is very important to determine the effectiveness of these products against natural enemies within the concept of IPM. In this study, the effect of leaf extract of *A. melanocarpa*, which is known to be of medical importance, on different developmental stages of an important biological control agent *T. evanescens* was investigated. The results obtained will create an infrastructure for the use of natural enemies and botanical products together in integrated control programs.

2. Materials and Methods

2.1. Plant material

The *A. melanocarpa* (Michaux) Elliott used in the study was purchased from the Aronia Sante company, which produces it in the Kırklareli province, Türkiye. The leaves of the purchased plant materials were dried and stored at 4 °C after grinding. The solid-liquid extraction method was used for the preparation of the leaf extract. Water was used as solvent for extraction (Tosun, 2009).

2.2. Parasitoid culture and bioassay

Egg parasitoid *T. evanescens* (Westwood, 1833) was collected from naturally parasitized *Ostrinia nubilalis* Hübner (Lepidoptera: Crambidae) eggs in Çukurova region, in Türkiye and reared on *Ephestia kuehniella* Zeller (Lepidoptera: Pyraidae) eggs under laboratory conditions. Parasitoids were cultured in a long daylight (16 h L: 8 h D) air-

conditioning chamber at 27 ± 1 °C and $70\pm5\%$ humidity. In experiments, *E. kuehniella* eggs were used as a host. Host eggs were sprinkled on egg cards with Arabic gum and placed in tubes along with single females of newly emerged *T. evanescens* females for 24 h. A drop of honey was placed on the edge of the egg card to feed the parasitoid. Egg cards with parasitized host eggs were immersed in *A. melanocarpa* leaf extract solutions (0.1%, 0.5% and 1%) and dH₂O (control) for 5 s at 24 h, 48 h, 72 h and 192 h after parasitism, corresponding to the egg, larvae, prepupal and pupal stages of *T. evanescens*, respectively (Battisti et al., 2020). Then, the egg cards were placed in air-conditioning chamber at 27 ± 1 °C and $70\pm5\%$ humidity and with a long daylight (16 h L: 8 h D). The parasitization, adult emergence and sex ratio of *T. evanescens* were evaluated. Each replicate consisted of 50 eggs and 6 replicates were done for each concentration.

2.3. Data analysis

Tukey test in SPSS 10.0 at the $P < 0.05$ level was used to calculate the toxicity of the *A. melanocarpa* leaf extract on different life stages of insects. Probit analysis was used to forecast LC₅₀ value (George and Mallery, 2019).

3. Results and Discussion

In the current study, *A. melanocarpa* leaf extract effectively killed the prepupal stage of *T. evanescens*. The insecticidal activity changed due to increasing concentrations of leaf extract solution. The prepupal stage of the parasitoid was more susceptible than other stages. Egg and pupal stages were more resistance than larval and prepupal stages (Egg LC₅₀: 0,772%, Pupa LC₅₀: 0,412% respectively). A 100% mortality rate was obtained by the leaf extract solution of *A. melanocarpa* in 1.0% for 24 h against prepupal stage of parasitoid. In this study, the larvae of parasitoid were tolerant to the toxicity of *A. melanocarpa* leaf extract compared to prepupal stage (LC₅₀: 0,133% and 0,075% respectively).

In this research, the most susceptible stage was determined to be the prepupal stage. The difference between the doses was found to be statistically significant in terms of adult, female and male emergence at the prepupal stage (Figure 3). Although the difference between the doses in terms of adult and female emergence in the egg, larval and pupal stages were statistically significant, the numerical decrease in the prepupal stage compared to the control was dramatic (Figure1-4).

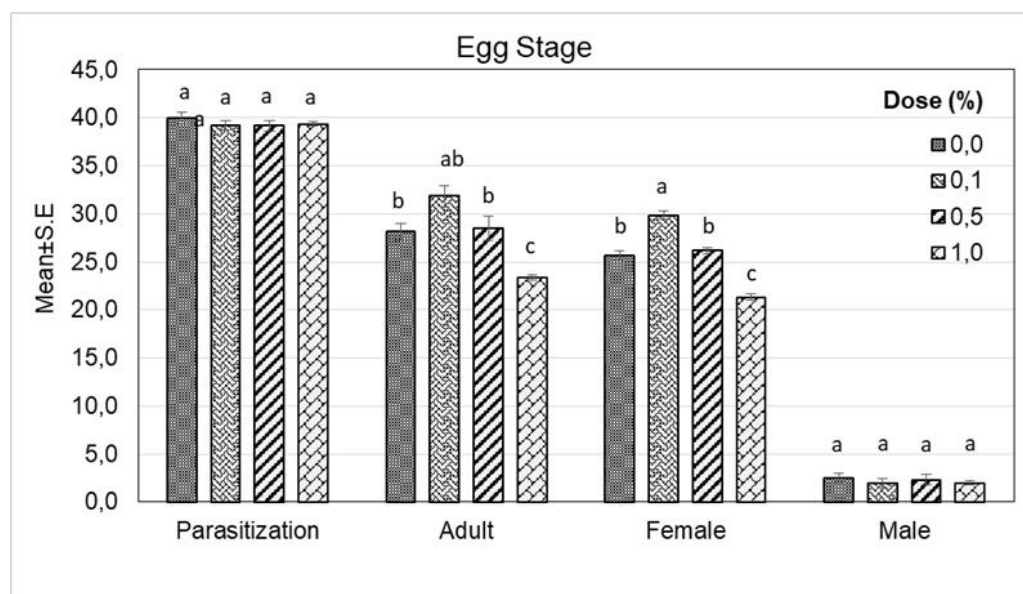


Figure 1. Mean percentages of parasitization, adult emergence and female emergence ($\pm$ SE) of *T. evanescens* egg stage after different dose application of *A. melanocarpa* leaf extract

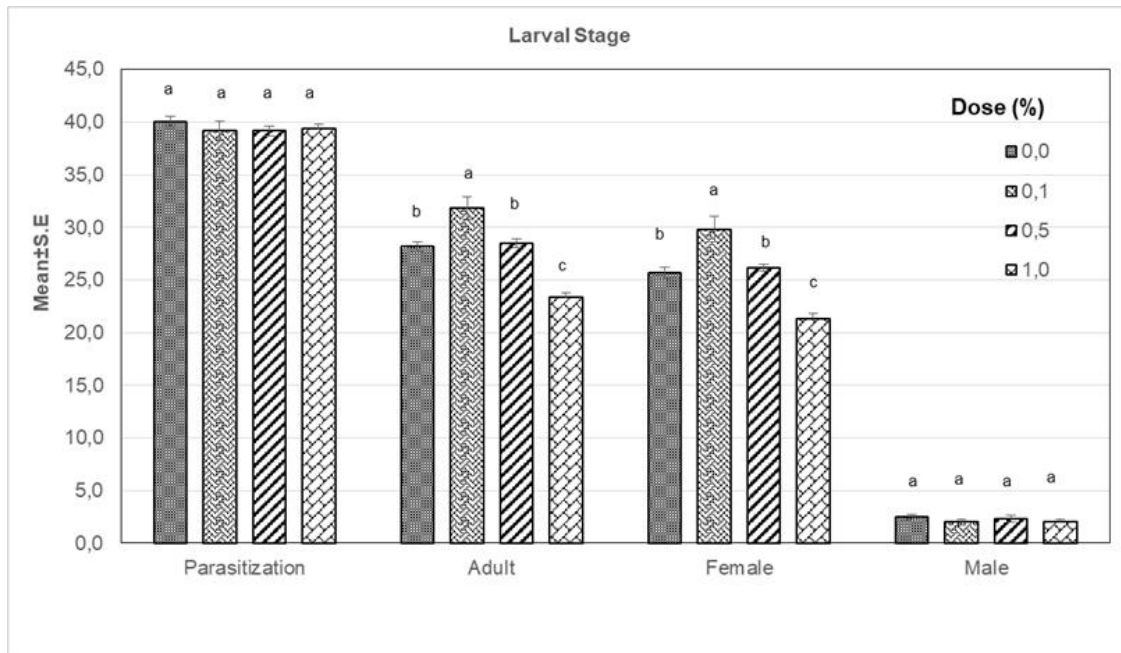


Figure 2. Mean percentages of percent parasitization, adult emergence and female emergence ($\pm$ SE) of *T. evanescens* larval stage after different dose application of *A. melanocarpa* leaf extract

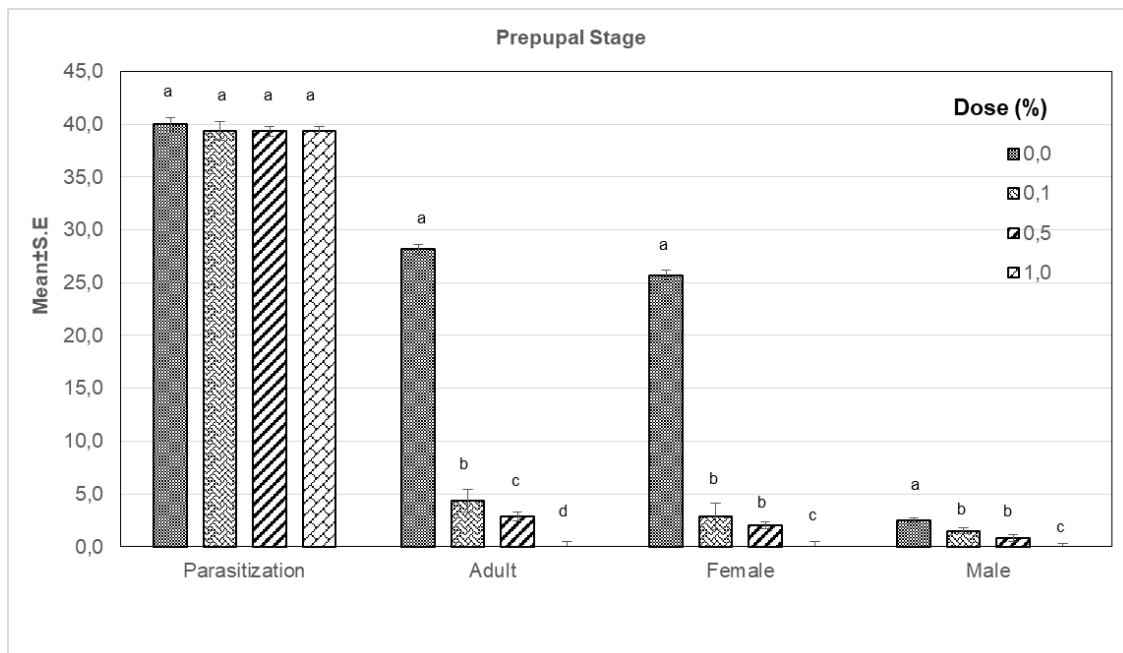


Figure 3. Mean percentages of percent parasitization, adult emergence and female emergence ($\pm$ SE) of *T. evanescens* prepupal stage after different dose application of *A. melanocarpa* leaf extract

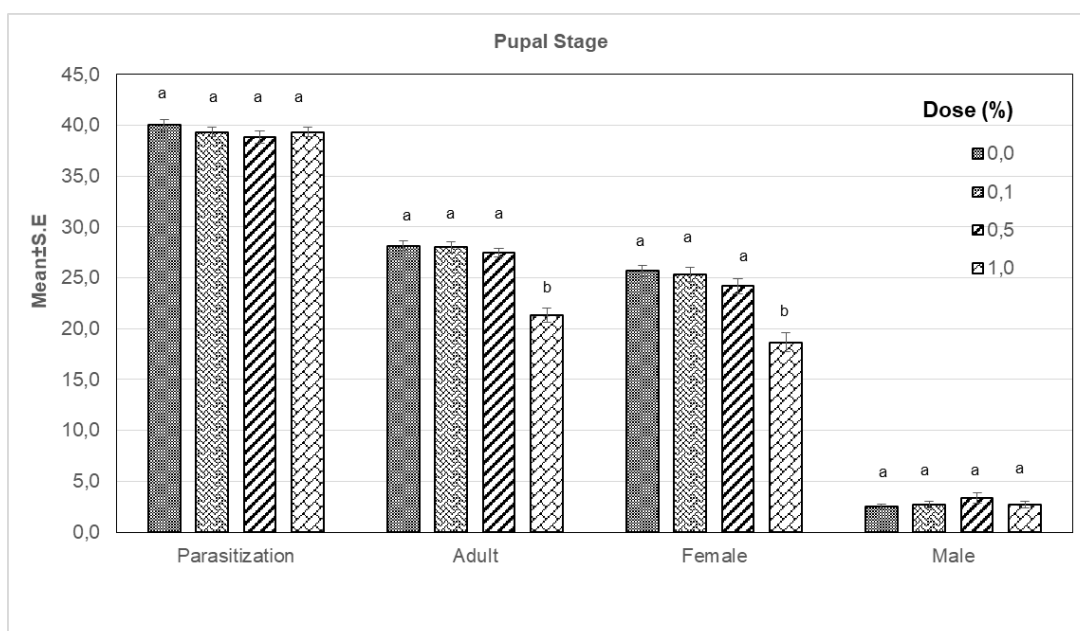


Figure 4. Mean percentages of percent parasitization, adult emergence and female emergence ($\pm$ SE) of *T. evanescens* pupal stage after different dose application of *A. melanocarpa* leaf extract

No type of pesticide is entirely safe. However, it is known that botanical pesticides are relatively safer than synthetics. There are various examples of extracts and essential oils of botanical pesticides and, in low doses, do not cause significant harm to natural enemies. Moreover, the effect may be based depending on the parasitoid type, active ingredient and dose (Tunca et al., 2012).

It is known that *Trichogramma* species are used effectively in biological control programs, and these species are polyphagous egg parasitoids (Koca et al., 2018). Unfortunately, beneficial insects are more sensitive than pests to the substances used in chemical control, and *Trichogramma* is one of them. Tavares et al. (2009) demonstrated that extracts obtained from Asteraceae selectively showed more insecticidal effect against *Trichogramma* sp.. In another study, it was shown that the essential oil of *Prangos ferulacea* reduced the development of *T. embryophagum* at the egg stage (Ercan et al., 2013). In the same study, it was determined that the pupal stage was more tolerant than the egg stage. Many studies are known to determine the effects of various plant products on *Trichogramma* species. Asrar et al. (2022)

were determined neem seed extract effect on *T. chilonis*. In another study, the insecticidal effect of *Annona muricata* plant extract on stored product pest, *Ephestia kuehniella* and biological control agent *T. evanescens* was investigated (Ata and Ercan, 2019). Monaj et al. (2020), determined the effect of coriander plant extract on the parasitization behaviour of *T. chilonis*. Gladenucci et al. (2020) investigated the selectivity and sublethal effects of some botanical extracts to pupal stage of *T. pretiosum*. Oudenhove et al. (2023) studied the non-target effects of ten essential oils on the *T. evanescens*. Tunca et al. (2016) were investigated the effect of azadirachtin, pyrethrum and d-Limonene on the parasitism rate of *T. pintoi*. They reported that the most susceptible stage of parasitoid was larval stage. In adult toxicity experiment, pyrethrum was found to be more toxic than azadirachtin. In our study, we determined that the most susceptible stage was the prepupal stage.

In integrated production systems, pest control is achieved using natural enemies like parasitoids and predators, but chemical or organic-based insecticide applications are necessary for complete control. It is desirable for plant-derived insecticides to be selective

for non-target organisms like natural enemies (Zanuncio and Lacerda, 2018). However, sometimes plant-derived products can negatively affect these beneficial insects. For example, Gonçalves-Gervazio and Vendramim, 2004 were found that Azadirachtin formulations reduced the emergence of *T. cacoeciae*. In another study, the applied oils did not prevent the parasitoid from completing its lifecycle, although it reduced the female longevity of *T. chilonis* (Packiam and Ignacimuthu, 2012). In our study, it was determined that *A. melanocarpa* leaf extract completely prevented the parasitoid from completing its life cycle when applied at the prepupal stage (1.0% dose).

4. Conclusions

This is the first study on the insecticidal effects of *A. melanocarpa* leaf extract on *T. evanescens*. The leaf extract of *A. melanocarpa* completely prevented adult emergence at a dose of 1.0% in the prepupal stage. Different life stages of insects have different sensitivities to crop products. In this research, the sensitivity or resistance of different life stages of the parasitoid to the relevant plant extract was determined to be different from each other. The results revealed the effects of this plant extract on beneficial insects. Therefore, in a biological control program to be planned against crop product pests, first the release of egg parasitoid, after the parasitoid overcomes the prepupal stage, the application of *A. melanocarpa* leaf extract will ensure the success of the program. Thus, combined but sequential application of the parasitoid and plant extract suppresses the population density of the pest.

Declaration of Author Contributions

The authors declare that they have contributed equally to the article. All authors declare that they have seen/read and approved the final version of the article ready for publication.

Declaration of Conflicts of Interest

All authors declare that there is no conflict of interest related to this article.

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