

Effectiveness of four release doses of two parasitoids species, *Trichogramma* (Hymenoptera: Trichogrammatidae) in some biological aspects of the lesser date moth *Batrachedra amydraula* Meyricke, 1916 (Cosmopteridae: Batrachedridae) on date palm trees in Basrah /Iraq.

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Abstract: Date palms are infested with various destructive insect pests, which cause major economic losses. One of these pests is the smaller date moth *Batrachedra amydraula* Meyricke, 1916. The study aimed to examine the effectiveness of two different *Trichogramma* egg parasitoid (*Trichogramma brassicae* Bezdenko, 1968 and *T. evanescens* Westwood, 1833) in the biological control of the lesser date moth *B. amydraula*. Each parasitoid species was examined at four different release doses (250, 500, 750, and 1000 parasitoid/tree). The findings revealed that all species of *Trichogramma* had a substantial effect, particularly at high doses, on the high parasitism of *B. amydraula* eggs, reaching 86.75 % for a dose of 1000 *T. evanescens* parasitoids/tree, with a significant difference reached 83.49 % at a dose of 1000 *T. brassicae* parasitoids/tree. The maximum rate of emergence of *T. evanescens* adults was at a dose of 1000 parasitoids/tree, which was much higher than *T. brassicae* reached 64.20 %. The response of absolute doses of *Trichogramma* females to *B. amydraula* spawning sites was evaluated, and the parasite's egg sites were determined according to tree height. The best parasitism rate was recorded for both *Trichogramma* species on trees whose height ranged from 1.5 m-2 m.

Key words: natural enemies, lesser date moth, *Trichogramma*, date palm, *B. amydraula*.

Učinkovitost štirih velikosti izpustitve parazitoidnih vrst iz rodu, *Trichogramma* (Hymenoptera: Trichogrammatidae) na nekatere biološke parametre manjšega dateljevega molja *Batrachedra amydraula* Meyricke, 1916 (Cosmopteridae: Batrachedridae) na palmah v Basri, Irak

Izvleček: Dateljevec napadajo različni škodljivci, ki povzročajo velike ekonomske izgube, med njimi je mali dateljev molj (*Batrachedra amydraula* Meyricke, 1916). Namen raziskave je bil preučiti učinkovitost dveh različnih parazitoidov jajčec iz rodu *Trichogramma* (*Trichogramma brassicae* Bezdenko, 1968 and *T. evanescens* Westwood, 1833) za biološki nadzor malega dateljevega molja. Vsaka parazitoidna vrsta je bila preučevana v štirih različno velikih izpustih (250, 500, 750 in 1000 parazitoidov na drevo). Rezultati so pokazali, da imata obe vrsti iz rodu *Trichogramma* precejšen učinek na jajčeca molja, še posebej ob večjih izpustih, kar je doseglo 86,75 % za izpust 1000 parazitoidov vrste *T. evanescens* na drevo, a značilno razliko 83,49 % pri izpustu 1000 parazitoidov vrste *T. brassicae* na drevo. Največje število odraslih osebkov vrste *T. evanescens* je bilo pri izpustu 1000 parazitoidov na drevo, ki je bilo veliko večje kot pri izpustu vrste *T. brassicae*, ki je doseglo le 64,20 %. Ovrednoten je bil odziv velikosti izpustov samic iz rodu *Trichogramma* na mesta razmnoževanja molja, kjer je bilo ugotovljeno število parazitiranih jajčec glede na višino drevesa. Največji obseg parazitizma je bil zabeležen za obe vrsti parazitoidov na drevesih, ki so bila visoka med 1,5 m-2 m.

Ključne besede: naravni sovražniki, mali dateljev molj, *Trichogramma*, dateljevec, *B. amydraula*.

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1 INTRODUCTION

Date palms (*Phoenix dactylifera* L.) are considered very important economic trees in different countries (Abass and Mazi, 2019). A large number of insect pests attacks palm trees in all their parts, which sometimes cause them serious and significant damage represented by the weakness of the palm tree, which leads to a reduction in its date productivity in terms of quality and quantity (Al-Jubouri, 2007; Al-Saedi, 2023). These damages also lead to the death of the palm tree. At the top of the palm tree, the pollen and fruits of the palm tree are exposed to many pests and insects that attack the flower pods before and after they open. They also attack the fruits in their various stages. One of these pests is lesser date moth (*Batrachedra amydraula* Meyrick, 1916) (Cosmopteridae: Batrachedridae), which usually cause fruit withering, pod breaking and falling. Several factors such as pests, weather conditions, and diseases affect health of palm trees. The lesser date moth (*B. amydraula* Meyrick) in Iraq is a serious pest that infects date palm trees (Downson and Aten, 1962; Levi Zada et al. 2011; Haldhar et al. 2017). The harmful stage of the pest is the larvae that feed inside the fruits, and also the larvae form networks around the flowers and later infect the fruit.

The use of chemical pesticides has affected the relationship between date palm pests and natural enemies. Thus integrated pest management (IPM) programs have become more complex because of increasing the pests (Latifian, 2001; Latifian and Zaerae, 2009; Latifian, 2012). Parasitoid of the genus *Trichogramma* are among the most common groups of natural enemies because they are relatively easy to rear and are among the most important egg parasitoid of the agricultural pest Lepidoptera. Biological control has been used as an alternative to chemical control in many South American and European countries, especially using egg parasitoids (Ivezic et al. 2022; Parra and Zucchim, 2004). The use of biological control methods to control *B. amydraula* is increasingly considered an essential management strategy within IPM. Parasitoids of the genus *Trichogramma* are among the most common groups of natural enemies because they are relatively easy to culture in the laboratory and are among the most important egg parasitoid of agricultural pests of the order Lepidoptera. *Trichogramma* parasitoids were used commercially as mass releases in more than 32 million hectares against more than 30 major insects worldwide (Wajnberg and Hassan 1994; Smith 1996; Ayvaz and Karaborklu, 2008; Pizzol et al., 2010; Pizzol, 2012). *T. brassicae* Bezdenko, 1968 was widely used worldwide for the control of lepidopteran pests as well as in egg control of *T. absoluta* (Meyrick, 1917) in some countries (Tissoli and Parra, 2001; Goncalves-Gervasio,

2003; Garcia et al. 2005; Medeiros et al., 2006; Desneux et al., 2010; Molla et al., 2011; Vasconcelos, 2013). In addition, Kuske et al. (2003) evaluated the effects of high releases of *T. brassicae* against the European corn borer *Ostrinia nubilalis* (Hübner, 1796) and was very successful in reducing the pest population and reducing yield losses. In Iran, *T. brassicae* is the most widespread species (Ebrahimi et al., 1998; Moezipour et al., 2008). Various factors can influence the effect of high releases of parasitoids, for example, host selection by parasitoids may be influenced by characteristics of both the host species and the host plant attacked by the pest (Chau and Mackauer, 2001; Desneux and Ramirez, 2009). Differences in parasitism depend on the host on which the parasitoids are reared and the effect of the size of the host born on natural parasitism rates of the host in the field, where female parasitoids usually accept host eggs of the same size or larger than the host (El-Wakeil, 2007; Nurindah et al., 1999). However Kolliker-Ott et al., 2003) reported no relationship between mass preference and performance of *T. brassicae* reared on the novel host *Ephestia kuehniella* Zeller, 1879 when parasitizing the target host of the European corn borer *Ostrinia nubilalis*. Otherwise, the effectiveness of *Trichogramma* release may depend not only on the biological characteristics of the parasitoid species or strains used, but also on their interactions with the pest plant (Tabone et al. 2010; Andrade et al. 2011; Yuan et al. 2012). *Trichogramma* is primarily used as a biocontrol agent through releases but the effect of *Trichogramma* generations growing within a crop can play an important role in the success of biological control programs (Mills, 2010). Torres et al., 2001) showed that the female parasitoid preferentially lays eggs in the upper part of the plant, followed by the middle and lower parts respectively. In the current study, the efficiency of four doses of these parasitoids in controlling *B. amydraula* Meyrick, 1916 was examined. The main goal of the study was to determine how *T. brassicae* and *T. evanescens* Westwood, 1833 impact the distribution of *B. amydraula* eggs on date palm trees and study the effect of tree height on the efficiency of parasitism.

2 MATERIALS AND METHODS

2.1 HOST PRODUCTION

Insects were collected from the University of Basrah, Date Palm Research Center (Basrah, Iraq) where the parasitoids, *Trichogramma brassicae* and *T. evanescens* were produced by the Mediterranean flour moth, *Ephestia kuehniella* (Lepidoptera: Pyralidae) in large quantities on flour. The Mediterranean flour moth was reared

in the laboratory on flour inside plastic cages (50 x 50 x 50 cm). All cage walls were covered with a piece of terry-cloth. Inoculated females of *E. kuehniella* were obtained by mating a male with a virgin female. Then, after one day the mating, *E. kuehniella* females were used in the experiments, and then the insects were placed in plastic cones, the base of which were covered with a terrycloth, through which the eggs are collected. Then the fertilized eggs were taken and placed in plastic containers (40 x 25 x 25 cm) filled with 2 kg of flour and were infested with *E. kuehniella* eggs. The moths begin to appear after they spread on the flour and moths continue to emerge for two months.

2.2 BREEDING THE PARASITE

The parasitoids, *Trichogramma brassicae* and *T. evanescens* (Hymenoptera: Trichogrammatidae) were produced in large quantities on the eggs of the Mediterranean flour moth, *Ephestia kuehniella*. Eggs of the Mediterranean flour moth *E. kuehniella* were pasted onto cardboard measuring 8 x 4 cm. Each card contained 250, 500, 750, and 1000 eggs. *T. brassicae* and *T. evanescens* are reared in glass containers with a height of 21 cm and a diameter of 10cm covered with muslin cloth by using rubber bands that are kept in place until use. Six cards of freshly collected eggs (1 day old) were placed in each glass jar along with 2 cards containing parasitic eggs that would hatch within 24 hours. *E. kuehniella* and *Trichogramma* species were reared at a temperature of 25 ± 2 °C, 70 % relative humidity (El-Dakroury et al., 2002).

2.3 THE EFFECT OF THE HOST LOCATION ON TRICHOGRAMMA PARASITISM AND ON *B. AMYDRAULA* EGGS

This study was conducted in Abu Al-Khasib area in Basrah Governorate, southern Iraq, in 2019. Halawi and Al-Sayer varieties were used. The parasitoids *T. brassicae* and *T. evanescens* were released in 2019 at the beginning of March. Four cards with doses of (250, 500, 750, and 1000 parasitoids/tree) were hung on palm branches at different heights (1.5-2 m), (2.5-3 m), and (3.5-4 m). The sample size was 10 branches (replicates)/ palm tree.

2.4 STUDY PARAMETERS

Presence of live larvae of *B. amydrula* on fruits whether they are fallen or not with the presence of silk tissue was recorded. Samples were collected and placed

in plastic bags for the purpose of examination in the laboratory. The parameters were: incidence and severity of the moth infection, the number of moth adults, the sex ratio, and the moth mortality (which should be corrected according to Henderson-Tilton's formula, 1955). The examination was conducted at two-week intervals from the beginning of March 2022 until the beginning of June 2023.

2.5 STATISTICAL ANALYSIS

The rate of parasitism results, adult emergence, sex ratio and adult longevity were analyzed statistically according to a randomized complete block design (CRBD) using one-way analysis of variance (ANOVA) and the results were compared by calculating the smallest difference. If significant differences were detected, comparisons were made using and significance of means was determined based on Duncan's test at $P \leq 0.05$. Statistical analysis was performed using SPSS Statistics for Windows version 21 (Al-Rawi and Khalafallah, 1980).

3 RESULTS

The results of releasing four doses of the parasite *Trichogramma brassicae* and *T. evanescens* to control the lesser date moth *B. amydrula* showed that there is a significance in the rate of parasitism of *T. evanescens* reached 86.75 %, 67.11 %, 55.32 % and 42.89 % at 1000, 750, 500 and 250 parasitoids/ tree respectively. These values were slightly higher than the rate of parasitism of *T. brassicae* which was recorded 83.49 %, 58.67 %, 47.33 % and 33.79 % at 1000, 750, 500 and 250 parasitoids/ tree respectively (Fig.1).

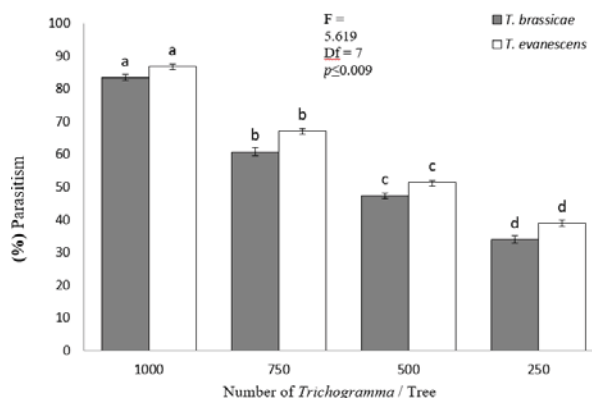


Figure 1: Parasitism according to the four doses of *Trichogramma brassicae* and *T. evanescens* on *B. amydrula* eggs.

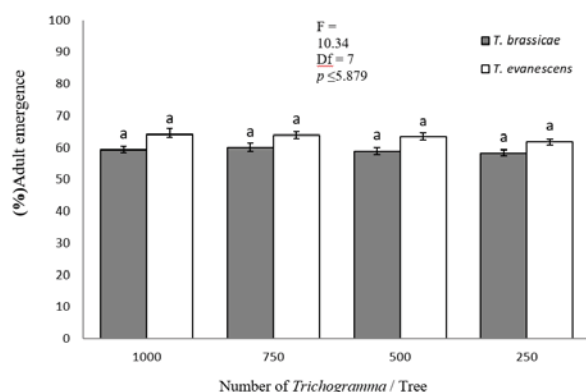


Figure 2: Adult emergence according to the four doses of *Trichogramma brassicae* and *T. evanescens* on *B. amydracula* eggs.

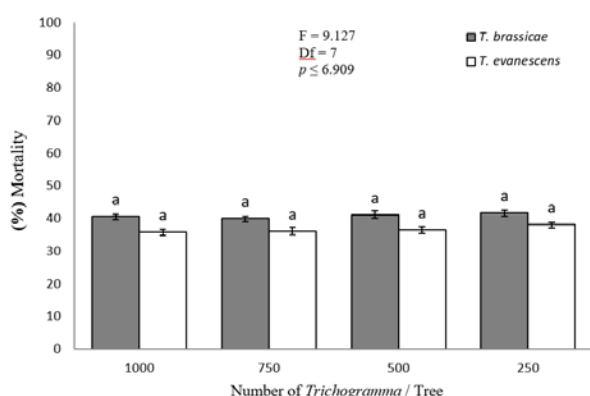


Figure 3: Mortality according to the four doses of *Trichogramma brassicae* and *T. evanescens* on *B. amydracula* eggs.

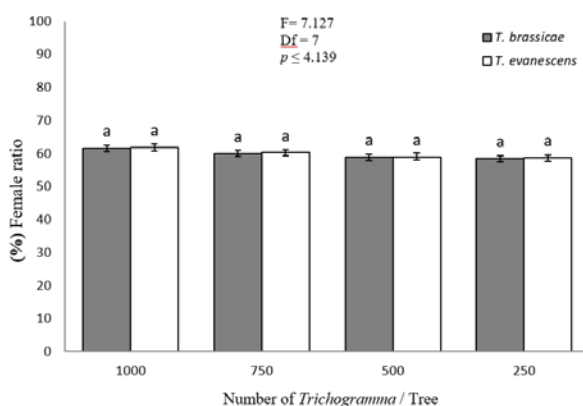


Figure 4: Female ratio according to the four doses of *Trichogramma brassicae* and *T. evanescens* on *B. amydracula* eggs.

However, there was no significance in the rate of emergence of adults of the *T. evanescens* parasite at the

doses of 1000, 750, 500 and 250 parasitoids/tree which were 64.2, 63.59, 63.45 and 61.83 % respectively. The results of adult emergence of *T. brassicae* parasite were slightly lower than *T. evanescens* after releasing 1000, 750, 500 and 250 parasitoids/tree, which were 59.4 %, 60 %, 58.85 % and 58.34 %, respectively (Figure 2).

The mortality rate of *T. evanescens* parasite reached 35.8 %, 36.05 %, 36.55 % and 38.17 % at doses of in the figure 3 they were 250, 500, 750 and 1000 parasitoids/tree, with a no significant in the figure 3 difference from the *T. brassicae* parasite, which recorded 40.6 %, 40 %, 41.15 % and 41.66 %, respectively, and this shows that the mortality rate of *T. evanescens* was lower than that of *T. brassicae*, but the difference between the mortality rates of these two parasitoid was significant when 1000 parasitoids/tree were released. The results showed that the female percentage of *T. evanescens* was slightly close to that of *T. brassicae* and was 61.8 %, 60.27 %, 59 % and 58.66 % for the doses 1000, 750, 500 and 250 parasitoids/tree for *T. evanescens* and was 61.6 %, 60.04 %, 58.83 % and 58.46 % for the doses 1000, 750, 500 and 250 parasitoids/tree for *T. brassicae*. However, the differences between the two parasitoid were all no significant in the figure 4 at the doses 1000 and 750 parasitoids/tree (Figure 4).

3.1 DISTRIBUTION OF THE PARASITE AND ITS PARASITISM ON THE PLANT CANOPY

The parasitism rate by the most preferred height of trees showed the behavior of the parasite *T. evanescens* on palm trees, a great parasitism on height 1.5-2 m and reached 58.95 %, 52.84 %, 50.42 % and 48.30 % for doses 1000, 750, 500 and 250 parasitoids/tree, compared to the parasite *T. brassicae* which reached 50.51 %, 42.70 %, 40 % and 39.99 % for doses 1000, 750, 500 and 250 parasitoids/tree. As for the height of trees 2.5-3 m, the parasitism rate was recorded lower than the previously mentioned height, with a significant difference, reaching 39.43 %, 28.26 %, 27.73 % and 20.04 % for the doses of 1000, 750, 500 and 250 parasitoids/tree for the *T. evanescens* parasite, and it reached 39.16 %, 36.92 %, 36 % and 31.79 % for the doses of 1000, 750, 500 and 250 parasitoids/tree for the *T. brassicae* parasite. While a significant difference was found for the parasitism of *T. evanescens* on tree height 3.5-4 m which reached 10.71 %, 18.90 %, 21.85 % and 22.66 % for doses of 1000, 750, 500 and 250 parasitoids/tree, as well as for the parasitism of *T. brassicae* which reached 10.33 %, 20.38 %, 24 % and 28.22 % for doses of 1000, 750, 500 and 250 parasitoids/tree (Figure 5).

4 DISCUSSION

The present study showed good efficiency of *Trichogramma brassicae* and *T. evanescens* in controlling the damage levels of the lesser date moth *B. amydracula*. Overall, *T. evanescens* showed the highest egg parasitism and thus appears to be more effective when compared with *T. brassicae* in biocontrol activity against the lesser date moth *B. amydracula*. Our results were comparable to those of Mohammad et al. (2011), who noticed that the lesser date moth infestation levels in Anbar Governorate were significantly reduced after two weeks of releasing 100, 200, and 300 *T. evanescens* or *T. principium* per palm tree, with higher efficiency for the parasite *T. evanescens*. Both parasitoid were effective in reducing the infestation of this pest on date palm fruits during both the Hababok and Jamri stages for all release rates. On the other hand, study by Alrubeai et al. (2014) demonstrated the effectiveness of the egg parasitoid *T. evanescens* in reducing the lesser date moth during the 2011 and 2012 seasons and showed that releasing the egg parasitoid at a rate of 500 and 1000 parasitoids/palm tree achieved a reduction of 55.06 % and 67.45 %, respectively, in the infestation rate and was a good option for controlling the lesser date moth as an alternative method of chemical control. Our research was examined two species of *Trichogramma* parasitoid at different heights of palm trees. In comparison, our results showed that the parasitism efficacy was higher than that reported by Alrubeai et al. (2014) who conducted it in central Iraq and focused on one height and one cultivar of palm trees using one species of *Trichogramma* parasitoid. In another study, Gameel et al. (2014) showed the effectiveness of releasing the egg parasitoid *T. evanescens* on the eggs of the lesser date moth *B. amydracula* at a rate of ten releases in envelopes (20,000 egg parasitoid/acre) which led to a significant reduction in the infestation after successive releases of the parasitoid to reach a high level (75.06 %) in the 2013 season, overall, the reduction rate ranged from 72.49 % to 75.97 % with an average of 73.74 % across all trials. Mohammad et al. (2011) found that the highest control efficiency obtained by *T. evanescens* parasitizing the lesser date moth was 70.8 % when used at a rate of three capsules per palm tree (capsules containing about 100 parasitized pupae). In another study, Alrubeai et al. (2014) found that releasing the egg parasitoid *T. evanescens* at a rate of 500 and 1000 parasitoids/palm tree achieved a reduction in the infection rate of 55.06 % and 67.45 %, respectively.

The emergence rate of *T. evanescens* adults was acceptable and slightly higher than that of *T. brassicae*. Otherwise, the size of the host eggs reared on different hosts appears to influence the biological characteristics of *Trichogramma* parasitoid (Greenberg et al. 1998, Broto-

djojo and Walter, 2006). This may be explained by the fact that *Trichogramma* reared on smaller hosts are less robust and perform less well than those reared on larger host eggs (Liu et al. 1998). On the other hand, studies on other plants have shown that different species of *Trichogramma* differ in their preference for *Tuta absoluta* eggs, for example Zouba et al. (2013) found that *T. absoluta* eggs were small in size and that the rate of egg parasitism by *T. bourarachae* 65.64 % was significantly higher compared to *T. cacoeciae* 57.05 %. Furthermore, studies indicate that larger host eggs can positively influence factors such as the percentage of parasitism, the number of parasitoid emerging per egg, lifespan, and sex ratio (Waage and Ming, 1984; Bai et al. 1992; Greenberg et al. 1998). In fact, the rate of emergence of adult *Trichogramma* can also vary with the size and quality of the host egg, the number of parasitoid developing in each egg, the period of development in the host egg and the temperature (Doyon and Boivin, 2005; Pratisoli et al., 2005). In this study, we obtained acceptable levels of control of the lesser date moth *B. amydracula*, despite reducing the release doses. The results showed an inverse relationship between the release dose and the number of parasitoids of *B. amydracula* eggs, as expected the highest parasitism rate was recorded for *B. amydracula* eggs, so these results suggest that releasing 400 parasitoids of both *T. brassicae* and *T. evanescens* per tree could be the most appropriate dose in this study.

Release doses of *Trichogramma* were also selected in a study by Cabello et al. (2009) where it was shown that releasing 75 adults per square meter every 3 or 4 days was very effective in reducing pest numbers. Previous studies, including Alsaedi (2021), have shown that the recommended doses of *T. brassicae* and *T. embryophagum* (Hartig, 1838) at a rate of (50, 100 and 200 adults/tree) depending on the pest infestation levels during the first weeks of the date fruit growing season can effectively reduce the number of *B. amydracula*. This study showed that parasitism of *T. brassicae* and *T. evanescens* was associated with the number of eggs laid by *B. amydracula* on different parts of the plant canopy and tree height, and that both species tended to parasitize eggs on shorter host trees. This suggests that selection by *B. amydracula* on this site provides a better place for egg laying by the order Lepidoptera. Or it may be because the wind affects the parasite, pushing it from the top to the bottom to fall on shorter trees. When looking at the different plant structures, the level of bleaching by *B. amydracula* was higher in the upper part of the short trees than in the other parts. In this regard, a similar pattern of parasitism on host plant parts was discussed by Romeis et al. (1998) for other *Trichogramma* species on maize, cotton and pea, these results indicated that *Trichogramma* adjusts

its parasitism to host distribution. It is known that one important plant characteristic that has a significant effect on parasitoid performance is the presence of non-glandular or glandular appendages (trichomes) (Oliveira et al. 2017). This is especially true for small parasitoid such as *Trichogramma* species that can be trapped by trichome secretions or have reduced walking speed on such surfaces (Romeis et al., 1998; Kennedy, 2003). *Trichogramma* is primarily used as a biological control agent by release but the effect of *Trichogramma* generations growing within the crop can play an important role in the success of biological control programs (Mills, 2010). Torres et al., 2001) showed that the female parasitoid preferentially laid eggs in the upper part of the plant, followed by the middle and lower parts respectively. This was in contrast to the finding in this study that the parasitoid preferred lower trees and may be due to wind speeds in relation to taller trees.

5 CONCLUSIONS

Both *Trichogramma brassicae* and *T. evanescens* demonstrated significant effectiveness in parasitizing *Batrachedra amydraula* eggs, particularly at higher release doses. The highest parasitism rate was observed with *T. evanescens* at a dose of 1000 parasitoids per tree, indicating that increasing the release dose enhances biological control efficiency. *T. evanescens* exhibited a higher adult emergence rate compared to *T. brassicae* at the highest release dose, suggesting a difference in their development and survival rates. The percentage of female parasitoids did not differ significantly between the two *Trichogramma* species, indicating that they have similar reproductive capacity. The parasitism rate was significantly greater in trees with heights ranging from 1.5m to 2m than in taller trees (3.5m to 4m), indicating that parasitoid efficiency is influenced by tree height and access to egg-laying sites. The study confirms that *Trichogramma* parasitoids are effective biological control agents against *B. amydraula*, with optimal performance at high doses and moderate tree heights. Future strategies should consider release dose and tree height for maximizing control efficiency.

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