Effect of Biological Characteristics of Two Species of *Trichogramma* on the Eggs of Lesser Date Moth, *Batrachedra amydraula* Treated with *Lawsonia inermis* Plant Extract

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Abstract

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This study aimed to evaluate the effects of treated and untreated *Batrachedra amydraula* Meyrick eggs with *Lawsonia inermis* (henna) on the parasitism rate, adult emergence rate, number of females, and adult longevity of two parasitoids, *Trichogramma brassicae* Bezdenko and *Trichogramma. evanescens* Westwood (Hymenoptera: Trichogrammatidae). The results obtained revealed that there were noticeable differences regarding the parasitism rate of the *Trichogramma* species. *T. brassicae* had the greatest parasitism rate (63%) on untreated *B. amydraula* eggs. However, when the eggs were treated with plant extract and water separately, the highest mortality rates caused by *T. evanescens* were 37.01 and 39.83%, by the two treatments, respectively. In addition, *T. brassicae* greatly outperformed the second species in terms of adult emergence rates when plant extract was not used to raise the larvae of *B. amydraula* 69.88%, followed by *T. evanescens* 65.02%. Compared to the other species, *T. brassicae* had a higher proportion of females. Additionally, *T. brassicae* lived longer compared to *T. evanescens* reaching 6.05 days. As far as we know, this is the first report investigating the ability of *T. brasicae* to biologically manage *B. amydraula* and reduce its population without the use of harmful pesticides. The results of the present study indicated that the use of *Trichogramma brassicae* and *T. evanescens* with *Lawsonia inermis* (henna) does not have a significant effect on the biological properties of *Trichogramma* when treated on *B. amydraula*.

Keywords: *Batrachedra amydraula*, date palm, *Trichogramma*, plant extract, *Lawsonia inermis*.

Introduction

The date palm, *Phoenix dactylifera* L. is an extremely important economic crop worldwide (Abbass & Maziel, 2019; Khalaf, 2013). World production in 2019/2020 reached 1132 million metric tons of dates (Statista, 2021). According to the Central Statistical Organization, CSO (2021), the output of dates from palm trees in Iraq has reached roughly 735,353 tons, with an average production of 66.7 kg/palm. The palm sector in Iraq was exposed to several problems that led to a decline in its numbers from 30 million palm trees to less than 15 million palm trees. In addition, many palm orchards were neglected, especially in Basra Governorate, which led to a clear increase the attack of this tree with some pests such as stem borers, dust mites and stem rot disease. The infestation rate with *Batrachedra amydraula* Meyricke insect was estimated in the range 70-100% in Basra orchards, especially in years when conditions are favorable for the insect (Fayyad *et al.,* 2022). Several insect pests attack palm trees in all of their parts, causing them sometimes serious and great damage that manifests as the palm's weakness and a decline in the quantity and quality of dates. The insect pest *Batrachedra amydraula* Meyricke, the lesser date moth (Cosmopterygidae: Lepidoptera) attacks date fruits and causing them to wilt and crack before falling (Al-Jubouri, 2007), and this pest is reported to be a harmful pest to date palm in Iraq (Levi Zada *et al.,* 2011). This pes has several generations per season, and its larvae eat within the fruit and weave webs around flowers, and yield loss may exceed 90% at some locations (Al-Jubouri, 2010).

Trichogramma parasitoids are frequently used in biological control programs all over the world; in the 1980s, 28 species were released in 28 nations to manage lepidopteran pests in annual and perennial crops, covering a total area of 32 million hectares (Hassan, 1997; Smith, 1996; van Lenteren, 2000). Plant extracts are used together with chemical pesticides in IPM programs for controlling pests without causing significant environmental harm. By using a variety of control measures that limit insect colonization of the crop, raise pest mortality death rates, or lower pest natality rates, modern integrated pest management (IPM) of crops, minimize crop damage and losses caused by pests (Bernal, 2010). IPM includes the application of selected pesticides and natural enemies such as *Trichogramma* species (Foerster, 2002; Parra, 2014).

Due to the lack of studies and research on the lesser date moth *B. amydraula* in Basrah Governorate, and the effect of plant extracts on *Trichogramma*, this knowledge can help in creating successful pest management programs using parasites and plant extracts, accordingly the current study aimed to assess the effect of spraying henna extract on palm trees and on the efficiency of two types of *Trichogramma* in parasitizing *B. amydraula* eggs.

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Materials and Methods

Insect pest and parasitoids rearing

All insects were collected from University of Basrah, Date Palm Research Center (Basrah, Iraq). The parasitoids *Trichgramma brassicae* and *T. evanescens* were found in large quantities on the eggs of the Mediterranean flour moth, *Ephestia kuehniella* (Lepidoptera: Pyralidae). The insect was reared in a climate chamber (25±2°C, 70% RH, 16hr:8hr L:D photoperiod) in the lab on wheat flour. Moths of *E. kuehniella* were collected every day and kept in oviposition cages measured 40 cm long by 18 cm wide and were covered with wire screens. The cages' eggs were used to rear the parasitoids. In this study, *T. brassicae* and *T. evanescens* were reared on eggs of the Mediterranean flour moth, *E. kuehniella* at Basrah University, in a climate chamber (25°C, 70% relative humidity, 16hr:8hr L:D photoperiod). Mated wasps that were 2 to 5 days old were used in the experiments. *Ephestia kuehniella* eggs were adhered to 6 × 4 cm cardboard cards, each containing 50 eggs. *T. brassicae* and *T. evanescens* were reared in glass jars 21 cm high and 10 cm in diameter, were covered with muslin material and held in place with elastic bands. Each glass jar included six cards of newly deposited eggs (one day old) and two cards containing parasitized eggs, which give rise to parasitoid adults within 24 hours. *E. kuehniella*, *T. brassicae*, and *T. embryophagum* were reared at a temperature of 25±2°C, 70% RH, and 16hr:8hr L:D photoperiod period.

Preparation of the plant extract

The leaves of the *Lawsonia inermis* (henna) plant were washed with water to remove the dust, and then placed in an electric oven at 25°C for drying, with continuous stirring to prevent them from rotting, for a period of three days. The samples were then kept in bags until used for extraction. For extraction, the leaves were ground using an electric grinder. Then, 20 g of *L. inermis* powder were dissolved in 200 ml of ethyl alcohol (70% ethanol) and placed in Soxhlet extractor for 8 hours at 60°C. The crude extract (filtrate) was the placed in a rotary evaporator. The concentrated raw extract was then placed in clean glass bottle and stored in the refrigerator at 4°C until use (Almansoori *et al.,* 2021).

Effect of *Trichogramma brassicae* and *T. evanescens* on the eggs of *B. amydraula* in the laboratory

A laboratory colony of adults of the parasitoids *Trichogramma brassicae* and *T. evanescens* parasitoids was prepared using 50 eggs/strip of *B. amydraula*, pasted on a rectangular card (6 x 8 cm) before the start of the experiments. 10 newly emerged females of the parasitoid were introduced into each glass vial (10 cm high x 2.5 cm wide) containing one egg parasitism card, with a small drop of honey added to one side to feed the parasitoid adults, and then incubated in a growth chamber, set at a photoperiod of 16 hr L:8 hr darkness, 70% relative humidity, and a temperature of 25°C. Female wasps were provided with a thin layer of honey solution (10%) as a diet. Ten replicates were used for each treatment. The rate of parasitism, proportion of females, adults emergence rate, and parasitism period were recorded.

Effect of plant extract on *B. amydraula* eggs in the laboratory

The spraying method was used to treat *B. amydraula* eggs with the henna plant extract, *L. inermis*. The egg cards (described above) were treated with three sprays of plant extract before parasitism with *Trichogramma* species, using a 250 ml hand sprayer. The control treatment was a spray with distilled water only, and the eggs were then exposed to parasitism by the two *Trichogramma* species, separately. Ten replicates were used for each treatment. The exposed eggs were then kept in the development room at 25°C, 70% relative humidity, and a 16 hr L: 8 hr D photoperiod. The overall parasitism rate for each wasp species, adult emergence rate, number of females, and adult longevity were documented.

Statistical design and analysis

All experiments were conducted under controlled laboratory conditions. Experiments followed a completely randomized design (CRD). Data obtained was analyzed using SPSS statistics for windows version 21 (SPSS 2012), and significance of means was determined based on LSD values at P=0.01 (Al-Rawi & Khalafallah, 1980).

Results and Discussion

The biological characteristics of *Trichogramma brassicae* and *T. evanescens* are summarized in Figures 1, 2, 3, 4, and 5. There were significant differences in the parasitism rates of the two *Trichogramma* species when *B. amydraula* eggs were exposed to wasps with or without a plant extract (F= 3.649; df= 8; P 0.008) (Figure 1-A). The results obtained indicated that the high rates of *T. brasicae* activity may be due to the characteristics of the species, its family range, its foraging ability, and its tolerance to changes in weather conditions such as temperature. It should be noted that the parasite's colony is newly formed, because it is a species collected from the local environment. Accordingly, the parasitic foraging ability and host preference of the natural enemy that distributes its presence to attack the most abundant prey (host species) (Hegazi *et al.,* 2019).

The mortality rate (%) of *T. evanescens* offspring was also affected by the treatment compared to the parasitoid *T. brassicae* individuals, regardless of whether *B. amydraula* eggs were treated with plant extract or not (Figure 2). The mortality of parasitic wasps also varied between the two *Trichogramma* species (F= 10.348; df= 8; P 0.011). On the other hand, the lowest mortality rate of parasitic wasps were observed when they were reared on *B. amydraula* eggs treated with a plant extract and reached 34.28% and 37.01%, respectively (Figure 1-B). The deadly impact of embryonic development on juvenile stages of insects can be explained by the reduction in specimen emergence in the F1 generation. In a previous study, individuals of *T. pretiosum* generated with *Couroupita guianensis*, may have disrupted embryonic development and immature stages by diffusing through the chorion of the egg, or they may have acted on the nervous system and convulsions were followed by paralysis and death in *Aedes albopictus* and *A. aegypti* (Parreira *et al.,* 2018).

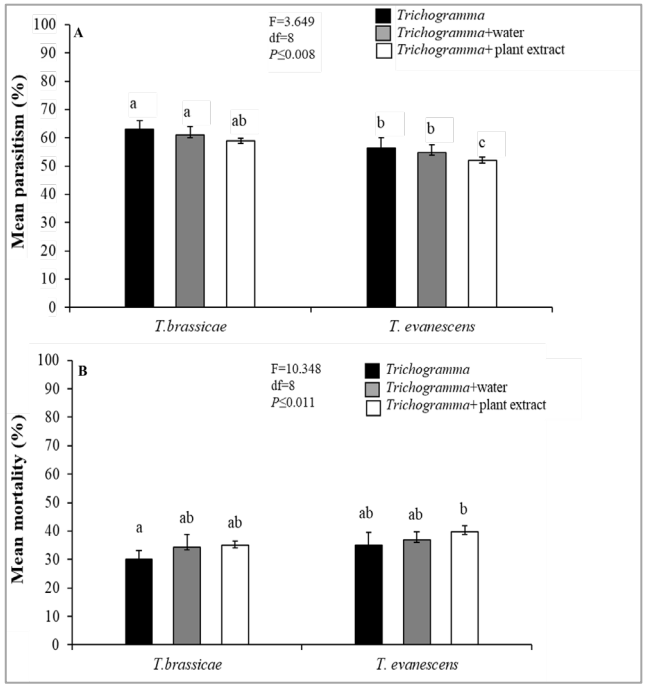

Figure 1. Comparison of *Trichogramma brassicae* and *T. evanescens* parasitism rates (A) and mortality rate (B) on *B. amydraula* eggs with and without Hanna plant extract treatment under laboratory conditions.

The percentage of adult emergence varied significantly between the two *Trichogramma* species (F= 9.127; df= 8; P 0.009). *T. evanescens* had the lowest rate of adult emergence independent from whether eggs treated with plant extract or not, whereas *T. brassicae* had the highest rate of adult emergence of 64.72% and *T. evanescens* had the highest rate of 62.99% on *B. amydraula* eggs treated with plant extract (Figure 2-A).

The highest female production rates were observed in *T. brassicae* reared on untreated eggs (84.47%) and plant extract-treated eggs (81.03%). On the other hand, the lowest percentage of females, with a significant difference (F = 5.361; df = 8; P = 0.013) was in *T. evanescens* (79.89%) when reared on untreated *B. amydraula* eggs (Figure 2-B).

The parasite egg is protected 1 day after parasitism by the chorion, the host, and the parasite, whereas in the prepupal and pupal stages, it is protected only by the chorion present in the host egg (Potrich *et al.,* 2017). This difference in protection may explain the lower toxicity of the essential oil.

Data presented in Figure 2-C indicated that *T. brassicae* lived significantly longer than other species when raised on eggs were untreated with plant extracts, reaching 6.05 days (F=13.119; df=8; P≥0.0001), whereas eggs treated with plant extracts reached 5.85 days. However, *T. brassicae* and *T. evanescens* adults did not show any significant difference in longevity as a result of the treatment with plant extract (Figure 2-C).

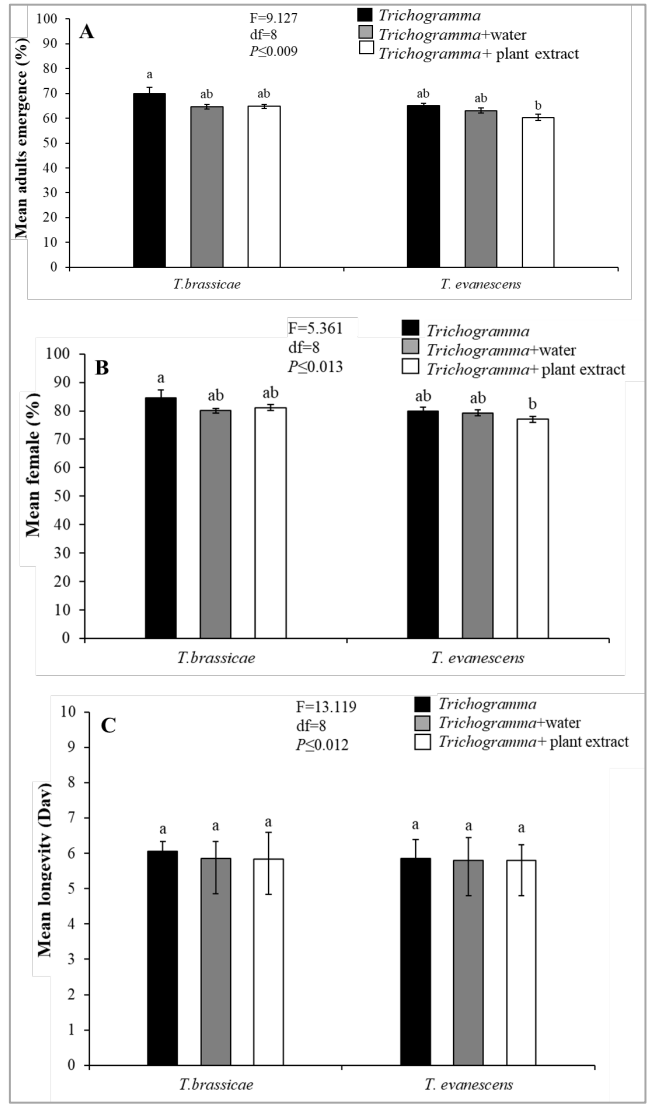

Figure 2. Adults emergence(A), female ratio (B) and longevity (C) of *Trichogramma brassicae* and *T. evanescens* reared on *B. amydraula* eggs treated with or without henna plant extract, under laboratory conditions.

The results obtained in this study were similar to previous reports (Khalif & Abd, 2017), which indicated that there was no effect of henna extract on the eggs of the house fly *Musca domestica* Linnaeus, as the incubation period of the eggs was one day at a concentration of 1250 mg/L All concentrations of henna extract used did not affect on the egg stage of the Dubas insect. (Al-Saedi, 2023). The role of the egg intruder in influencing the reduction of the *B. amydraula* bug infestation on the date palm arises from the biological instinct to lay parasitic eggs inside the *B. amydraula* eggs, nest inside them, and feed on their internal contents, which results at the end of its role in maturation and development. The results of this study somewhat agreed with the conclusion reached by Gameel *et al.* (2014), who suggested that the introduction of *T. evanecence*, as an egg parasite, led to a decrease in *B. amydraula* infestation rates, which fell from 7.22% to 2.00% four years later, which also agrees with another report (Ali *et al.,* 2010). According to Richards *et al.* (2010), specialist parasitoids such as *T. galloi* are less

sensitive to changes in host chemistry than generalist parasitoids because of their similar emergence, longevity, and parasitism in any generation of *T. galloi* exposed to *Eois nympha* (Lepidoptera: Geometridae) *C. sinensis, M. piperita*, *O. vulgare*, *S. aromaticum* and *T. vulgare.* Additionally, none of the *E. nympha* had an impact on the F2 generation's reproductive characteristics, indicating that they won't have any adverse impacts on future *T. galloi* generations similar to those brought on by synthetic pesticides on this species (Costa *et al.,* 2014).

It can be concluded from this study that in recent years, the lesser date moth *B. amydraula* has become a major and important pest in palm fields in some palm growing areas in Iraq. The plant extracts of the biocontrol agent contributed to reducing the infection rates of *B. amydraula* to below the economic damage threshold. This study also showed that the two species of wasps *T. evancences* and *T. brassicae* were able to successfully parasitize *B. amydraula* eggs as herbivores and reduce the presence of *B. amydraula* on date palms. No direct and specific negative effect of henna extract was detected on *Trichogramma* species biological parameters such as longevity, adult emergence, parasitism rate, and mortality rate. The findings of this study encourages the use of *T. brassicae* because it may lead to desirable results for integrated management of *B. amydraula* which reduces the use of chemicals and thus improve food safety and environmental quality.

الملخص

الساعدي، غزوان فيصل ورامز مهدي العبد السعيد. 2025. تأثير الصفات الحيوية لنوعين من الدبابير التابعة للجنس *Trichogramma* على بيض حشرة *Batrachedra amydraula* المعالجة بمستخلص نبات الحناء (*Lawsonia inermis*). مجلة وقاية النبات العربية، 43(4):517−521.

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هدفت هذه الدراسة إلى تقييم تأثير بيض حشرة *Batrachedra amydraula* Meyrick المعالج وغير المعالج بمستخلص نبات الحناء (*Lawsonia inermis*) على معدل التطفل، معدل ظهور البالغات، عدد الإناث وطول عمر البالغات لاثنتين من الدبابير المتطفلة *Trichogramma brassicae* Bezdenko و *T. evanescens* Westwood (Trichogrammatidae : Hymenoptera). أظهرت النتائج المتحصّل عليها وجود فروق معنوية فيما يتعلق بمعدل التطفل لنوعي *Trichogramma* المدروسة، إذ أبدى المتطفل *T. brasicae* أعلى معدل تطفل على بيض *B. amydraula* غير المعالج بالمستخلص النباتي وبلغ 63%. أما عند معاملة بيض الحشرة بالمستخلص النباتي والماء بشكل منفصل، فإن أعلى معدلات النفوق الناجمة كانت للطفيلي *T. evanescens* إذ بلغت 37.01 و 39.83%، على التوالي. بالإضافة إلى ذلك، تفوق *T. brassicae* بشكل كبير على النوع الثاني للطفيلي المدروس من حيث معدلات ظهور البالغات عندما لم يتم استخدام المستخلص النباتي على بيض *B. amydraula* إذ بلغ 69.88%، يليه *T. evanescens* وبلغ 65.02%. بالإضافة إلى ذلك، عاش *T. brassicae* لفترة أطول مقارنة بـ *T. evanescens* حيث وصل إلى 6.05 يوم. يعدّ هذا التقرير الأول حول استخدام المتطفل *T. brasicae* لإدارة *B. amydraula* حيوياً وتقليل أعدادها دون استخدام المبيدات الحشرية الضارة. أشارت نتائج الدراسة أيضاً إلى أن استخدام المتطفلين *T. brassicae* و *T. evanescens* مع مستخلص الحناء (*L. inermis*) ليس له تأثير معنوي على خواصهما الحيوية عند تربيتهما على بيض الحشرة *B. amydraula*.

كلمات مفتاحية: مستخلص نباتي، نخيل التمر، *Lawsonia inermis*، *Trichogramma*، *Batrachedra amydraula*.

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