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Impact Temperatures on the Biological Characteristics of the Bio-parasitoid *Trichogramma* spp. Reared on the Eggs of the Lesser Date Moth *Batrachedra amydraula* (Meyrick).

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Abstract

Lesser date moth *Batrachedra amydraula* presents an important threat to the sustainability of date palm in Iraq. This study evaluated two potential egg parasitoids *Trichogramma evanescens* and *T. brassicae*, through comparative laboratory and field assessment of their biological performance. Experiments examined thermal responses at 25, 30 and 35°C across Sayer, Hillawi and Khadhrawi date palm cultivars, measuring parasitism, emergence, embryo mortality and sex ratio. Results indicated a clear thermal optimum at 30 °C where *T. brassicae* achieved 80.11% parasitism and *T. evanescens* 79.93% on Hillawi cultivar. At 35°C, performance declined sharply to below 55% parasitism. *T. evanescens* showed highest emergence (64.2%) and lowest mortality (35.8%) at 30 ^{o}C, whereas *T. brassicae* exhibited high embryonic mortality (49.43%) at 35°C. Field observations revealed peak activity in April on Khadhrawi cultivars, while Hillawi showed the highest biological compatibility with a female biased sex ratio of 57 to 62.5%. The study identifies 30 ^{o}C as the optimal temperature for mass rearing and field release, and recommends *T. evanescens* as the most effective agent for IPM programs in southern Iraq, with April as the best release window before extreme summer temperatures. Overall, The integration of laboratory and field data demonstrates that temperature strongly governs parasitoid efficiency, with 30 ^{o}C representing a critical threshold for maximizing biological potential against *B. amydraula*, while heat stress beyond this limit reduces effectiveness and should be considered in climate-adapted integrated pest management strategies.

Keywords: *Batrachedra amydraula*, *Trichogramma spp*., Biological control, embryonic mortality, parasitism.

Introduction

Date palm (*Phoenix dactylifera* L.) plays a strategic role in agricultural and ecological systems for arid and semi-arid zones around the globe. In the Middle East and North Africa especially, date palm cultivation is essential for sustainability efforts. Date palm growing along Shatt Al-Arab basin in Iraq's south eastern governorate Basra has been part of local heritage and economy for many years. Due to this special ecosystem and microclimate, conditions are suitable for growing high quality cultivars which may have promising commercial value. Unfortunately, various insect pests threaten date palm plantation production, and the most important of these insects is lesser date moth *Batrachedra amydraula* (Meyrick) (Abd *et al.* 2025). This pest presents a major economic threat to date palm growers because of its voracious feeding behavior on the fruit during early stages of development (Hababouk and Kimri stages). This can lead to dried fruits that drop from the tree prematurely and result in significant financial loss if effective management strategies are not implemented (Latifian, 2020 ; Al-Naabi *et al*. 2023). Losses can exceed 50% of total yield in extreme cases. For many years now, controlling this pest has mainly depended on the use of traditional synthetic pesticides. Even though these chemicals can work for a short time, their widespread and careless use has led to serious harm to the environment (Abdifitah *et al.* 2026).. This has caused significant problems in Shatt Al Arab, including soil and water pollution, negative effects on positive organisms, and the creation of pesticide-resistant insects. Therefore, IPM practices need to be adopted immediately because biological control methods with Trichogramma egg parasitoids are an excellent and sustainable method to use for pest control (Bueno *et al.* 2023). These parasitoids are extremely effective at reducing pest populations during their most vulnerable stage at the egg which prevents any damage from being done before the fruit is even produced.This method is not only good for the environment but also really flexible for use in field release initiatives (Navik and Richa, 2018; Satyanarayana *et al.* 2024). This method is set to be a game changer in 2024.The field efficiency of biological control agents is not static rather it is the product of a complex interaction between the parasitoids genetic traits and surrounding environmental factors primarily temperature. Temperature serves as the fundamental driver for all biological and physiological processes in insects, determining developmental speed, longevity, and host-seeking capability, as well as directly influencing the sex ratio of the resulting progeny (Ana-Cristina *et al.* 2020). In a thermally fluctuating environment like Southern Iraq where temperatures transition from moderate spring conditions to

extreme summer heat stress understanding the thermal plasticity of these parasitoids is essential for the success of control programs (Ramos Aguila *et al.*2023 ; Alary *et al.* 2026). In addition to climatic factors the host plant cultivar plays a pivotal role in the success of the parasitism process. Morphologic, physical, or chemical traits of different date palm cultivars, including Sayer, Hillawi, and Khadrawi, may create differences in the attraction of pest species for oviposition or may affect the micro climate surrounding the egg. These variations then impact the parasitoids ability to locate the host and develop within. Despite a wealth of global literature on the genus *Trichogramma*, there remains a significant gap in local research comparing the species *T. evanescens* and *T. brassicae* when reared on a natural host (*B. amydraula*) under varying thermal pressures across different date palm cultivars in the Basra environment (Alrubeai *et al.* 2014 ; Al-Musafir *et al.*2022 ; Al-Saedi, 2025). Thus, this research was conducted to provide a thorough and in depth investigation into how three different temperatures 25°C, 30°C and 35°C affect the biological characteristics of both species of these parasitoids. The objectives are to define the optimal temperature range for their activity, to see how their activities change when the host plant is grown from different cultivars. Therefore, this provides an accurate scientific database to aid in determining the best time to release them in the field of-date palm orchards, both for protection of the crop and for maintaining the ecological integrity of the area.

Materials and Methods

Study Area and Site Description

Field trials conducted in private date palm orchards located in the Shatt Al-Arab district of Basrah Province, Southern Iraq selected a geographic site representing the environmental conditions of date palm cultivation that experienced large seasonal variability in the magnitude and duration of temperature fluctuations. The research evaluated three commercially grown varieties of the date palm Sayer, Hillawi, and Khadhrawi. Homogeneous trees (10–15 years old) with similar heights were selected ensuring they were subjected to uniform agricultural practices (irrigation, fertilization and pruning) throughout the study period to minimize external variables.

Host Insect Rearing and Maintenance

The initial source of lesser date moth *B. amydraula* was derived from larval-infested fruit bunches located within a defined study area, these samples were then transported to laboratory facilities and maintained within wooden boxes with fine mesh cover at 25±2°C and 60±5% RH

using the same experimental methodologies. Parcels of freshly laid less than 24h eggs were collected for parasitic experimentations thereafter to periodically ensure that the maximum suitability was provided to each host material (Al-Naabi et al 2023).

Parasitoid Sources and Acclimatization

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 *E. kuehniella* were pasted onto cardboard measuring 8 x 4 cm. Each card contained 50 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 Trichgramma species were reared at a temperature of 25 ± 2 °C, 70 % relative humidity (El-Dakroury *et al.*, 2002).

Thermal Experimental

To evaluate the biological characteristics of the parasitoids under varying thermal pressures, the following steps were implemented, three constant temperatures (25°C, 30°C, and 35°C) were maintained inside programmed growth chambers with a precision of ±0.5°C. One hundred fresh Lesser Date Moth eggs (categorized by cultivar) were fixed onto small paper cards and exposed to a mated pair of parasitoids for 24 hours. Five replicates were assigned to each treatment (Parasitoid species × Temperature × Cultivar). Following the exposure period cards were transferred to clean test tubes and kept in incubators to monitor embryonic development (Mohammad *et al.* 2015).

Assessment of Biological Parameters

The following detailed biological indicators were recorded parasitism rate (%) calculated based on the number of eggs that turned black due to the development of parasitoid larvae. Adult emergence percentage (%) calculated by counting the exit holes left by emerging adults in the host eggs. Embryonic mortality rate (%) calculated as the percentage of parasitized (blackened) eggs that failed to produce emerging adults. Sex ratio emerged adults were examined under a

stereomicroscope to differentiate females from males (based on antennal morphology) to calculate the female progeny percentage.

Statistical Analysis

The data were analyzed using a Randomized Complete Block Design (RCBD) with a factorial experimental framework. Analysis of variance (ANOVA) was used to analyze the effects of the temperature, the parasitoid species, the date palm cultivars, and their interactions on the biological parameters measured after the parasitism of the date palm trees. To determine the significance of differences between the means, a Duncans Multiple Range Test was used with a significance level of ($P \leq 0.05$). All statistical analyses were performed using the Statistical Package for Social Sciences (SPSS) software, version 23.

Results

Parasitism efficiency and the interaction between temperature and cultivars

The findings presented in Table (1) indicate a significant impact of temperature on the parasitism rates of both bio-parasitoid species. The temperature of 30°C was statistically superior (significance level a) compared to other thermal regimes. At this optimal temperature *T. brassicae* recorded its highest parasitism rate at 80.11% on the Hillawi cultivar, followed closely by *T. evanescens* at 79.93% on the same cultivar (Figure 1). When the temperature was increased to 35°C a significant reduction in parasitism efficiency occurred (level b) with rates fluctuating between 56.88% and 62.64% reflecting the negative impact of heat stress on female activity. Conversely, the lowest parasitism rates (level c) were recorded at 25°C reaching a minimum of 48.95% for *T. brassicae* on the Khadhrawi cultivar. As observed in Table (1) the Hillawi cultivar provided a more conducive environment for parasitism compared to Sayer and Khadhrawi, although the differences between cultivars were less pronounced than the variations caused by temperature.

Table (1): Interaction effect of temperature and date palm cultivars on the parasitism rate (%) of *Trichogramma* spp. on *B. amydraula* eggs.

Parasitoid Species	Temperature (°C)	Sayer (Mean ± SE)	Hillawi (Mean ± SE)	Khadhrawi (Mean ± SE)
T. evanescens	25°C	51.34 ±1.2 c	52.61 ±0.9 c	50.15 ±1.1 c
	30°C	78.45 ±0.8 a	79.93 ±0.5 a	77.20 ±1.0 a
	35°C	58.12 ±1.5 b	59.73 ±1.3 b	56.88 ±1.4 b
T. brassicae	25°C	49.88 ±1.1 c	50.43 ± 1.2 c	48.95 ±0.8 c
	30°C	79.12 ±0.7 a	80.11 ±0.6 a	78.45 ± 0.9 a
	35°C	61.20 ±1.8 b	62.64 ±1.5 b	60.10 ±1.7 b

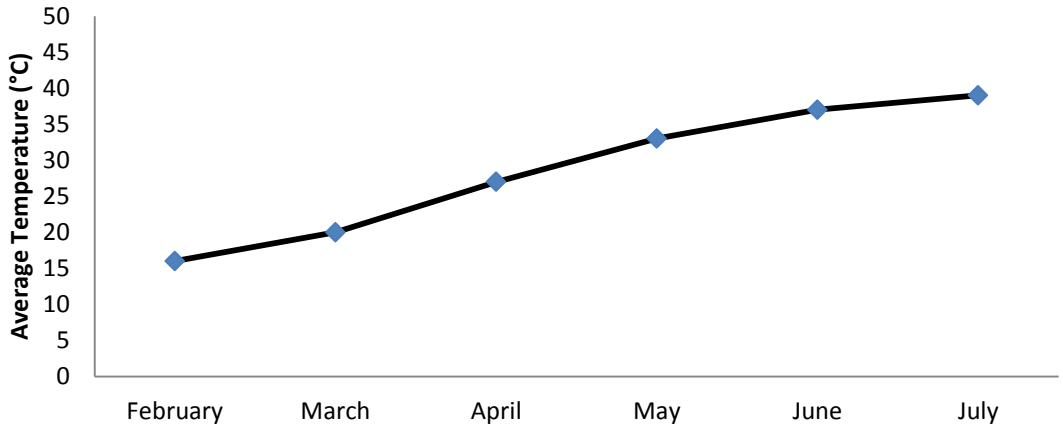

Figure 1: Average Monthly Temperatures in Basrah Governorate from February to July.

Biological Performance

Table (2) illustrates the biological characteristics related to the completion of the life cycle within the host egg. The results showed a qualitative superiority for *T. evanescens* at the optimal temperature 30°C achieving the highest adult emergence rate of 64.20%. This value was statistically significant (level a) outperforming *T. brassicae* which recorded 58.85% at the same degree. The relationship between embryonic mortality rates and emergence efficiency appears to be reverse. The lowest statistical embryonic mortality rate was recorded at 30°C for *T. evanescens* (35.80%). In comparison, both species exhibited high levels of embryonic mortality (50.85% and 49.43% respectively) in the high temperature (35°C) treatment which contributes to low levels of attack success by these natural enemies in field conditions during intense summer heat.

Table (2): Biological performance (Adult Emergence % and Mortality %) of *Trichogramma* spp. at various constant temperatures.

Parasitoid Species	Temp (°C)	Adult Emergence (%)	Mortality Rate (%)
T. evanescens	25°C	54.12 ±1.1 b	45.88 ±0.9 b
	30°C	64.20 ±0.8 a	35.80 ±0.7 c
	35°C	49.15 ±1.4 c	50.85 ±1.2 a
T. brassicae	25°C	52.33 ±1.0 b	47.67 ±1.1 b
	30°C	58.85 ±0.9 ab	41.15 ±0.8 bc
	35°C	50.57 ±1.5 c	49.43 ±1.4 a

Progeny Sex Ratio

Data from Table (3) indicate that all treatments produced offspring progeny with a female biased ratio, this is an economically desirable trait for biological control programs. *T. brassicae* reached the maximum percentage of female progeny (62.50%) on the Sayer cultivar at 30°C. Similarly, temperature analysis indicates that temperatures of both 25°C and 30°C maintained high female percentages and statistically consistent female ratios (level a) without significant differences between these temperatures, however a temperature of 35°C significantly decreased female percentages (level b) at all replication of cultures tested with *T. evanescens* producing the lowest ratio of 57.85% on the Kadhrawi cultivar. This decrease may be due to the fact that thermal stress may impact the viability of fertilized (female) and unfertilized (male) eggs differently (more so on fertilized eggs).

Table (3): Influence of temperature and date palm cultivars on the Sex Ratio (Female %) of the progeny.

Parasitoid Species	Temperature (°C)	Sayer (%)	Hillawi (%)	Khadhrawi (%)
T. evanescens	25°C	60.12 a	61.33 a	59.45 a
	30°C	60.45 a	61.41 a	60.05 a
	35°C	58.20 b	59.08 b	57.85 b
T. brassicae	25°C	61.15 a	61.88 a	60.30 a
	30°C	62.50 a	62.10 a	61.55 a
	35°C	59.10 b	60.02 b	58.70 b

General Synthesis of Numerical Data

By correlating the three tables it is evident that the triple interaction between parasitoid species, temperature, and host-plant cultivar plays a pivotal role in the success of biological control. While *T. brassicae* possesses a slightly higher parasitism capacity at high temperatures (Table 1), *T. evanescens* excels in embryonic viability and adult emergence (Table 2), making it the more sustainable species for the thermally variable environment of Shatt Al-Arab district-Basrah governorate-Iraq.

Discussion

The data show that the two types of insects achieved 80 percent parasitism at 30°C on Hillawi. Palm trees have an ideal temperature for their excellence in metabolic efficiency. However, at 35°C the two species became critically separated. *T. evanescens* had a high degree of physiological plasticity with a 64.2% rate of emergence associated with *T. brassicae* shigh embryonic mortality and sensitivity to high temperatures. The cultivar of Hillawi provided a protective micro climate from thermal degradation resulting in increased success of the parasitoid. Based on these data, *T. evanescens* should be used for IPM programs in southern Iraq during the Golden Biological Window in April, in which there is an alignment of temperature and physiological optimal. The timing allows pest control to be maximized prior to the arrival of extreme summer heat waves during June. The results from the study by Gameel *et al*., (2014) support the efficacy of *T. evanescens* through a series of long term field trials during which *T. evanescens* was released at 20,000 parasitized eggs/feddan in early May. These trials showed an average of a 73.74% reduction in the level of infestation of *B. amydraula*. The similar result from this study provides the conclusion that selecting *T. evanescens* is an effective strategy to minimize the impact of lesser date moth infestations and subsequently fruit drop. According to the findings of Mohammad *et al.*, (2011), the results of our study are congruent with one another confirming that *T. evanescens* would be the best species available to use for controlling pests in southern Iraq using biological pest control methods IPM. There is also an assurance that using *T. evanescens* will result in high quality crop production while providing agricultural producers with assurance about the safety of their environment as well as the health of humans living close to agricultural land. In addition, Mohammad *et al.* (2011) found that when releasing *T. evanescens* at a release rate of 3 capsules per palm it provided a maximum control efficacy of 70.8%. Analysis of efficiency of parasitism with thermal response the (Table 1) peak of the rate of

parasitism at approximately 30 degrees celsius is most likely due to an increase in the females activity level and host searching behaviour. At the same time we believe that the decrease in the rate of parasitism which was apparent at 35°C was the result of the effects from heat stress induced oxidative damage that reduced the average longevity and reproductive cycle of the female wasps. This accounts for the complete failure of parasitoids in Basrah during the very hot summer months, when the temperatures are usually over the upper limit of their thermoregulation. In the study performed by Khethani *et al.* (2013), the developmental biology of the parasitoid Trichogrammatoidea lutea was examined using six constant temperatures (18, 21, 24, 27, 30, and 35°C) in combination with three different lepidopteran host eggs. From this research it was determined that *T. lutea* was unable to complete its development at a temperature of 35°C using any of the three host species. It is evident from this study that a temperature of 35°C represents a critical thermal barrier that halts the development of many *Trichogramma* species. This underscores the vital importance of selecting the appropriate timing for field releases, ensuring they occur away from the peak summer heat. Emergence, Mortality, and Physiological plasticity the data indicated that *T. evanescens* had an advantage over other species in terms of both emergence and mortality (Table 2). This leads us to believe that *T. evanescens* has a greater degree of Physiological Plasticity enabling the *T. evanescens* embryo to withstand thermal stress while residing in its host egg. The increased mortality level at 35°C may not only be due to deaths of individual embryos; it also may reflect that the host egg *B. amydraula* was desiccated by superheated air in which they were incubated. The resultant hardening of the chorion of the host egg will create a mechanical barrier, thus preventing larvae from feeding upon or emerging from their' host eggs. Mohammad *et al.* (2015) demonstrates that *T. evanescens* is a highly effective bio-agent against the lesser date moth, achieving a peak parasitism rate of 94.4% within an optimal thermal range of 27°C. Findings indicate that temperature significantly dictates life stages, with longevity stretching to 35 days at 15°C and contracting to 7 days at 33°C. Population stability and sex ratio although the sex ratios value remained stable (Table 3), the small decrease in the temperature at 35°C supports the hypothesis that female embryos formed from fertilized eggs have a greater environmental sensitivity than male embryos. Maintaining a female ratio of about 60% is an effective indicator of whether or not these parasitoids will successfully establish populations in palm orchards when released at the correct biological window. A study conducted in Iran by Tabebordbar *et al.* (2022) confirms that temperature is the decisive factor in determining the biological efficiency of the *T. euproctidis* parasite, as 32.5°C

was recorded as the optimal temperature for parasitism, female production, and survival. Life table indices showed a positive relationship between temperature rise and the intrinsic rate of increase up to a certain threshold, before decreasing at extreme heat 40°C. The Hillawi cultivar was found to be more suitable for the parasitoid species compared to the other cultivar's characteristics like the fruit is likely to provide some level of shelter from dry winds, thus creating a suitable micro-climate for the parasitoid. The Khadhrawi cultivar has greater levels of attractiveness towards the pest however, there may also be certain chemicals like tannins or resins present in this cultivar in greater concentrations than in the Hillawi cultivar that could hinder the parasitism of the pest by the parasitoid. Therefore, in order to effectively manage the pest within this cultivar, we will have to release higher concentrations of parasitoids within this cultivar. In a study he conducted Firake and Khan (2014) showed that daily temperature fluctuations and short term thermal shocks adversely affect the survival of *Trichogramma* parasites in terms of emergence, fecundity and longevity. In the same regard, Mehrnoosh *et al*. (2021) confirms that the efficiency of *T. brasicae* follows a non-linear relationship with temperature, peaking at 30°C before declining at higher thermal regimes. The results highlight the high performance of the parasite in tropical and warm conditions, while maintaining a biased sex ratio across different temperatures. Thus, these bioclimatic models enable optimization of mass rearing protocols and precise field release timing to ensure maximum control effectiveness.

Conclusion

The study found that 30°C is the optimal temperature for maximum parasitism and emergence, and is therefore recommended for mass rearing of *Trichogramma*. *T. evanescens* performed better than *T. brassicae*, especially under heat stress at 35°C, showing higher thermal tolerance. Host cultivar influenced effectiveness, with Hillawi showing the best compatibility, while Khadhrawi required higher release rates due to greater infestation levels. All treatments showed a female-biased sex ratio (57–62%), indicating good potential for stable field populations. Overall, April was identified as the best release period in southern Iraq, as field temperatures align with optimal parasitoid activity.

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تأثير درجات الحرارة على الخصائص الحيوية لطفيل البيوض *Trichogramma spp.* المربّى على بيض حشرة الحميرة *Batrachedra amydraula* (Meyrick)

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الخلاصة

تُعد حشرة الحميرة *Batrachedra amydraula* من الآفات المهمة التي تهدد استدامة زراعة نخيل التمر في العراق. هدفت هذه الدراسة إلى تقييم نوعين من متطفلات البيوض هما *Trichogramma evanescens* و*T. brassicae* من خلال مقارنة أدائهما الحيوي مختبرياً وحقلياً. شملت التجارب دراسة استجابة النوعين لثلاث درجات حرارة (25 و30 و35°م) على أصناف نخيل التمر الساير والحلاوي والخضراوي، مع تقييم نسبة التطفل ونسبة خروج الحشرات الكاملة ونسبة موت الأجنة ونسبة الجنس. أظهرت النتائج وجود درجة حرارة مثلى واضحة عند 30°م، حيث سجل *T. brassicae* أعلى نسبة تطفل بلغت 80.11%، في حين سجل *T. evanescens* نسبة تطفل بلغت 79.93% على صنف الحلاوي. وعند 35°م انخفضت كفاءة التطفل بشكل ملحوظ إلى أقل من 55%. كما حقق *T. evanescens* أعلى نسبة خروج للحشرات الكاملة (64.2%) وأدنى نسبة موت (35.8%) عند 30°م، بينما أظهر *T. brassicae* أعلى نسبة لموت الأجنة بلغت 49.43% عند 35°م. وبينت الملاحظات الحقلية أن أعلى نشاط للمتطفلات سجل خلال شهر نيسان على صنف الخضراوي، في حين أظهر صنف الحلاوي أعلى درجة من التوافق الحيوي مع المتطفلات، مع نسبة جنس منحازة للإناث تراوحت بين 57 و62.5%. وتحدد الدراسة درجة حرارة 30°م بوصفها الدرجة المثلى للإكثار الكمي والإطلاق الحقلي، كما توصي باستخدام *T. evanescens* بوصفه العامل الحيوي الأكثر كفاءة ضمن برامج المكافحة المتكاملة للآفات في جنوب العراق، مع اعتماد شهر نيسان موعداً مثالياً للإطلاق قبل حلول درجات الحرارة الصيفية المرتفعة. وتؤكد النتائج أن درجة الحرارة تعد عاملاً حاسماً في تحديد كفاءة المتطفلات، إذ تمثل 30°م الحد الحراري الأمثل لتعظيم قدرتها على مكافحة عثة تمر النخيل الصغرى، في حين يؤدي تجاوز هذا الحد إلى انخفاض فعاليتها، الأمر الذي ينبغي مراعاته عند تطوير استراتيجيات المكافحة المتكاملة المتكيفة مع الظروف المناخية.

الكلمات المفتاحية: حشرة الحميرة، متطفلات Trichogramma ، المكافحة الحيوية، موت الاجنة، التطفل.