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PRODUÇÃO VEGETAL

# INFLUENCE OF AMBIENT CONDITIONS ON THE PARASITIZATION OF *Anagasta kuehniella* EGGS BY *Trichogramma pretiosum*

Fernando Bellesini Vigna[1*], Murilo Gaspar Litholdo[2], Elaine dos Santos Guidetti Karlinke[2], Kênia Rezende Silva[2], Alexandre de Sene Pinto[2]

[1]Faculdade de Ciências Agrárias e Veterinárias - Câmpus de Jaboticabal - UNESP, Via de Acesso Professor Paulo Donato Castelane Castellane S/N - Vila Industrial, Jaboticabal - SP CEP: 14884-900.
[2]Centro Universitário Moura Lacerda, Av. Dr. Oscar de Moura Lacerda, 1520 – Ribeirão Preto – SP, CEP: 14076-510
*** Corresponding author:** Fernando Bellesini Vigna, febelvi@gmail.com

**ABSTRACT:** This study investigated the parasitism rate of *Trichogramma pretiosum* (Hymenoptera: Trichogrammatidae) on *Anagasta kuehniella* eggs (Lepidoptera: Pyralidae) in various crops, including maize, crotalaria, and sorghum, while considering diverse weather conditions. Conducted over multiple time points from 2016 to 2018, bioassays were designed with meticulous detail. In each assay, 100 blue cardboard strips (measuring 10 x 0.6 cm, with each strip containing 50 *A. kuehniella* eggs at the edge) were strategically placed on 20 randomly selected plants within a 5-meter radius from the central point of the field. Egg strips installed at 6:00 am and continued throughout the day, with replacements every two hours until 6:00 pm. Fresh strips were then placed overnight, starting from 6:00 pm until the subsequent morning at 6:00 am. The release of *T. pretiosum* took place at 6:00 am in the central region of the experimental area. It was observed that the parasitism rate exhibited its highest values when the relative humidity exceeded 40%. Parasitism peak typically occurred between 12:00 pm and 2:00 pm. Interestingly, parasitism rates were notably lower immediately after the release of parasitoids and during night-time hours. This suggests that *T. pretiosum* requires a certain time for dispersion and initiation of parasitism, with substantial activity decline during nocturnal periods.

**KEYWORDS:** ecology, biological control, egg parasitoid, Hymenoptera, parasitoid.

# INFLUÊNCIA DAS CONDIÇÕES AMBIENTAIS NO PARASITISMO DE OVOS DE *Anagasta kuehniella* POR *Trichogramma pretiosum*

**RERSUMO:** Este estudo investigou a taxa de parasitismo da vespa *Trichogramma pretiosum* (Hymenoptera: Trichogrammatidae) em ovos da traça *Anagasta kuehniella* (Lepidoptera: Pyralidae) em diferentes culturas, incluindo milho, crotalária e sorgo, e sob condições climáticas variadas. Os bioensaios foram conduzidos em vários momentos de 2016 a 2018, com cada ensaio envolvendo a colocação de 100 tiras de papelão azul (cada tira medindo 10 x 0,6 cm e contendo 50 ovos de *A. kuehniella* na borda) em 20 plantas selecionadas aleatoriamente dentro de um raio de 5 metros de um ponto central no campo.

As tiras de ovos foram instaladas às 6:00 da manhã e substituídas a cada duas horas até as 18:00, com novas tiras colocadas durante a noite até a manhã seguinte às 6:00. A liberação de *T. pretiosum* ocorreu às 6:00 da manhã no centro da área experimental. A taxa de parasitismo foi mais alta quando a umidade relativa estava acima de 40%. O pico de parasitismo geralmente ocorreu entre 12:00 e 14:00. Curiosamente, o parasitismo foi significativamente mais baixo logo após a liberação dos parasitoides e durante a noite. Isso sugere que o *T. pretiosum* precisa de algum tempo para se dispersar e iniciar o parasitismo, e que sua atividade de parasitismo diminui significativamente durante as horas noturnas.

**PALAVRAS CHAVE:** ecologia, controle biológico, parasitoide de ovos, Hymenoptera, parasitoide.

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## INTRODUCTION

Biological control stands as a pivotal component in the strategic management of pests, focusing on facilitating the natural self-regulation of pest populations without using pesticides. This approach ensures that pest numbers remain below the economic damage threshold by focusing on their natural enemies (Parra, 2006).

Microhymenopterans belonging to the genus *Trichogramma* (Hymenoptera: Trichogrammatidae) have gained global recognition as effective biological control agents against lepidopteran pests in diverse agricultural and forestry settings. Their popularity is attributed to their ease of rearing in alternative hosts and their efficiency in parasitizing insect pest eggs (Botelho, 1997; Parra, 1997; Haji et al., 1998).

In fact, *Trichogramma*-based biological control programs are operational in 49 countries, with inundative releases of this parasitoid carried out on more than 21 million hectares annually. Literature estimates; however, vary between 16 and 32 million hectares per year, covering 28 different crops and involving 28 *Trichogramma* species (Hassan, 1993; Smith, 1996; Hassan, 1997; Parra & Zucchi, 2004).

In Brazil, *Trichogramma* holds a prominent position as the most widely used natural enemies in various crops (Parra & Junior, 2019). *Trichogramma galloi* Zucchi, for instance, is used to parasitize *Diatraea saccharalis* (Fabr.) (Lepidoptera: Crambidae) over two million hectares of sugarcane crops. *Trichogramma pretiosum* (Riley), known for its expansive host range among species produced at large scale, parasitizes *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) and *Chrysodeixis includens* (Walker) (Lepidoptera: Noctuidae) larvae over 250,000 hectares of soybean and maize crops, Tuta absoluta (Meyrick) (Lepidoptera: Gelechiidae) over 1,500 hectares of tomato crops, and *Lasiothyris luminosa* (Razowski & Becker) (Lepidoptera: Tortricidae) over 1,000 hectares of grapevine crops.

However, the success of *Trichogramma* spp. releases relies on a comprehensive understanding of the parasitoid ecology and its interactions with the intended host (Carvalho et al., 2014). Therefore, laboratory studies examining the parasitism potential of the chosen *Trichogramma* species are imperative before their application in commercial releases (Parra et al., 2002). Due to the potential compromise of observed success under unfavourable conditions, tests assessing the parasitoid in relation to environmental parameters should be conducted.

Temperature emerges as the most critical abiotic element, influencing the length of the parasitoid life cycle, parasitism rate, sex ratio, and longevity (Hoffmann and Hewa-kapuge, 2000; Molina et al., 2005). Various factors, including abiotic variables such as temperature, precipitation, and light, can impact the effectiveness of *Trichogramma* parasitism in the field after release (Cónsoli & Parra, 1994; Pinto & Parra, 2002; Pinto et al., 2003).

Despite extensive studies, there is still a need for further exploration into the behaviour of the genus *Trichogramma* and the variables that could influence its field effectiveness. Consequently, the current study aims to assess the parasitism rate of *T. pretiosum* on *Anagasta kuehniella* (Zeller) (Lepidoptera: Pyralidae) eggs in various crops, including maize, crotalaria, and sorghum, under diverse weather conditions.

## MATERIAL AND METHODS

### Site Location

All bioassays were carried out at the 'Moura Lacerda' University of Ribeirão Preto, SP. Environmental conditions during experiments, local temperature, and relative humidity were meticulously recorded using data loggers in experimental areas. The prevailing climate is temperate subtropical, with annual mean temperature of 21ºC and annual precipitation of 1,500 mm. The field area is located between 21º 9' 30.902" South and 47º 46' 45.109" West. Meteorological data of the experimental area were collected during experiments (Table 1).

**Table 1.** Monthly meteorological data collected during experiments carried out in Ribeirão Preto - SP.

| Month/Year | Pressure | Tmax | Tmin | Tmed | RH | Precipitation | DR | Insolation |
|---|---|---|---|---|---|---|---|---|
| Mar-16 | 944.5 | 31.4 | 20.2 | 24.6 | 79.3 | 132.9 | 17 | 209.0 |
| May-17 | 945.0 | 28.0 | 16.5 | 21.2 | 77.1 | 113.4 | 5 | 214.5 |
| Feb-18 | 942.3 | 30.5 | 19.7 | 24.0 | 77.4 | 86.8 | 12 | 186.4 |
| Mar-18 | 942.2 | 32.2 | 20.6 | 25.5 | 73.9 | 55.7 | 10 | 246.8 |
| Jun-18 | 947.7 | 29.1 | 15.1 | 21.1 | 63.6 | 0.0 | 0 | 201.8 |
| Aug-18 | 947.1 | 28.8 | 14.2 | 20.4 | 62.8 | 39.7 | 7 | 233.3 |

Pressure: atmospheric pressure; Tmax: maximum temperature; Tmin: minimum temperature; Tmed: mean temperature; RH: relative air humidity; DR: number of rainy days

**Insect rearing**

The insect species used in experiments were cultured in laboratory under controlled environmental conditions. This included maintaining temperature of 25 ± 1 °C, relative humidity (RH) of 70 ± 10%, and photoperiod of 12 hours of light and 12 hours of dark.

*T. pretiosum* specimens were obtained from the Insect Biology Laboratory at ESALQ-USP, Piracicaba, SP, Brazil. These were reared in *A. kuehniella* eggs, with maintenance procedures following the method proposed by Parra (2010). It is noteworthy that the *T. pretiosum* colony was composed solely of females, reproducing through thelytokous parthenogenesis, as documented by Pinto & Stouthamer (1994).

**First parasitoid release evaluation**

The study incorporated three distinct assays, conducted on March 05 and 15, 2016, and May 03, 2017. Transgenic maize (*Zea mays* L.) expressing the Cry1F toxin was cultivated during these periods. In 2016, maize was planted with 75 cm row spacing and density of 5-6 plants per linear meter, whereas in 2017, row spacing was expanded to 90 cm. No pesticides were administered throughout the experimental period.

The field experiment was designed to demarcate a circular area within the field using ten 20-cm tall wooden stakes, forming a radius of 5 m. Each stake supported two cardboard rectangles (15 x 10 cm), each adorned with five light-blue cardboard strips (10 x 0.6 cm). This configuration resulted in ten egg strips per stake, culminating in 100 strips per area. Each strip bore consisted of 50 sterile *A. kuehniella* eggs fixed with 50% gum Arabic.

Approximately 5.000 *T. pretiosum* adults emerged within the previous 12-24 hours and were released at the centre of this designated area around 6:00 am. These adults had been nourished solely on pure honey. The egg-bearing strips were replaced bi-hourly until 6 pm, with the final set of fresh eggs left in place until 6 am the following day. Strips containing A. kuehniella eggs were replaced every two hours until 6:00 pm, with the last strip with fresh eggs left in place until 6:00 am of the following day.

All egg-bearing cardboard strips were labelled with the time of parasitism and then placed in 2-cm diameter, 10-cm high flat-bottomed glass tubes, each sealed with plastic film. For the 2013 assays, these tubes were housed in a climate-controlled chamber, maintained at 27 ± 1ºC, 70 ± 10% humidity, and 14-hour photophase. The 2017 assay, however, was kept at a slightly lower temperature of 25 ± 1ºC under similar humidity and photophase conditions.

Six days after parasitism, each egg strip (approximately 50 eggs per strip) was scrutinized under a stethoscope microscope to quantify total and parasitized (dark) eggs.

**Second parasitoid release evaluation**

Five distinct assays were conducted, with parasitoid releases performed on February 17, March 13, March 16, June 1, and August 30, 2018. Throughout these experiments, no pesticides were applied.

The initial assay on February 17, 2018, assessed parasitoid release in a crotalaria crop (*Crotalaria juncea* L.) planted on December 18, 2016. Subsequent releases on March 13 and 16, 2018, were conducted in fields of transgenic maize expressing the Cry1F toxin, planted on December 16, 2017. On June 1, 2018, releases were simultaneously performed in two separate areas: one in a sorghum field (*Sorghum bicolor* L. (Moench)), planted on January 31, where non-emergent parasitoids were scattered on the ground, and the other in maize, planted on March 26, 2018, where emerged parasitoids were released. This maize plot also had a subsequent release on August 30, 2018.

A circular area with 5 m of radius was designated for the study within each field. On 20 randomly chosen plants, each with about 1.5 m in height, rectangular cardboard pieces (15 x 10 cm) were fixed. These rectangles bore five light blue cardboard strips (10 x 0.6 cm), each with roughly 50 *A. kuehniella* eggs adhered to the edge using a plant-based adhesive. This configuration yielded a total of 100 egg-adorned strips per area.

Around 6:00 am, at the centre of each area, approximately 5.000 *T. pretiosum* adults were released. These adults, who had emerged within the previous 12-24 hours, had been fed on pure honey.

The sole exception was the test carried out on June 1, 2018, where non-emerged parasitoids were scattered on the ground, and emergence was later confirmed. Egg-bearing strips were replaced every two hours until 6:00 pm, with the final set of fresh eggs left in place until 6:00 am of the following day.

All egg-bearing cardboard strips were labelled with time of parasitism, then placed in 2-cm diameter, 10-cm high flat-bottomed glass tubes, each sealed with plastic film. During experiments, parasitized egg-bearing strips were slightly moistened and housed in a climate-controlled chamber, maintained at 25 ± 1ºC, 70 ± 10% humidity, and 14-hour photophase.

Six days after parasitism, each egg strip (approximately 50 eggs per strip) was scrutinized under stereoscopic microscope to quantify total and parasitized (dark) eggs.

### Statistical analyses

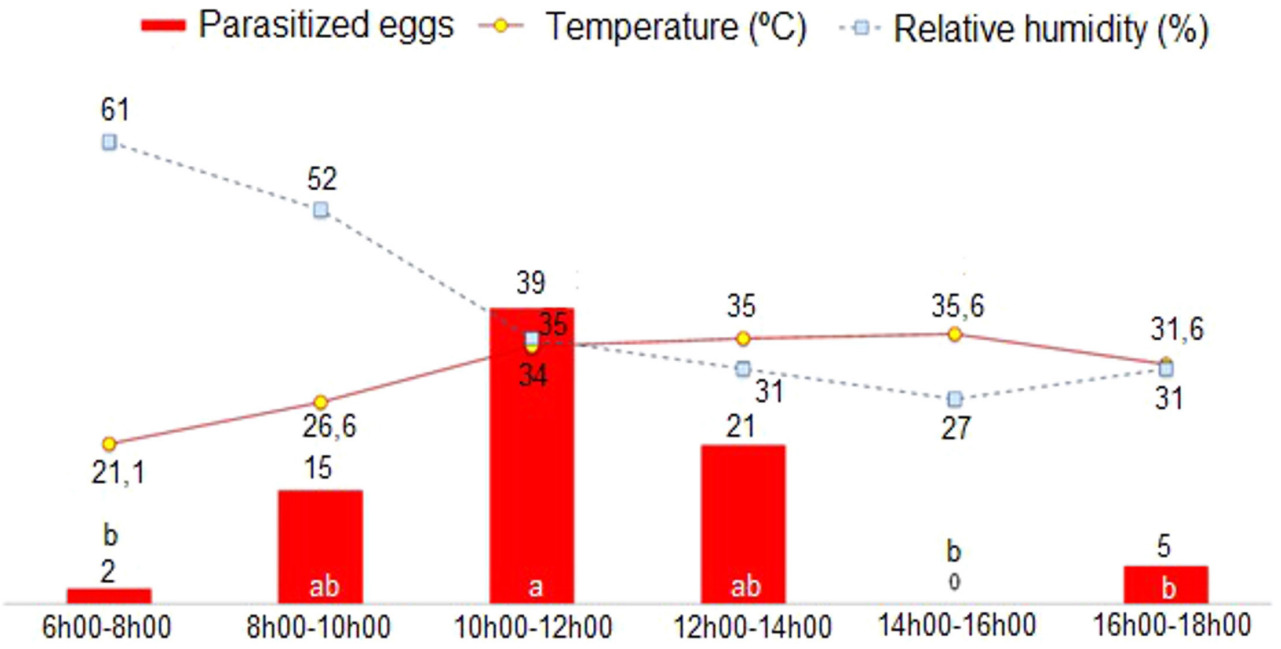
Statistical analyses were performed using the R software (R Core Team, 2020). Mean values were computed for all data sets, and each parasitism period was evaluated using analysis of variance (ANOVA) with the F-test at 5% significance level. To compare mean values in all evaluation days, the first experiment used the Duncan's multiple range test at 5% level. The second experiment used the Tukey's honestly significant difference test at 5% level. However, the displayed graphs represent the total counts of parasitized eggs.

## RESULTS AND DISCUSSION

### First parasitoid release bioassay

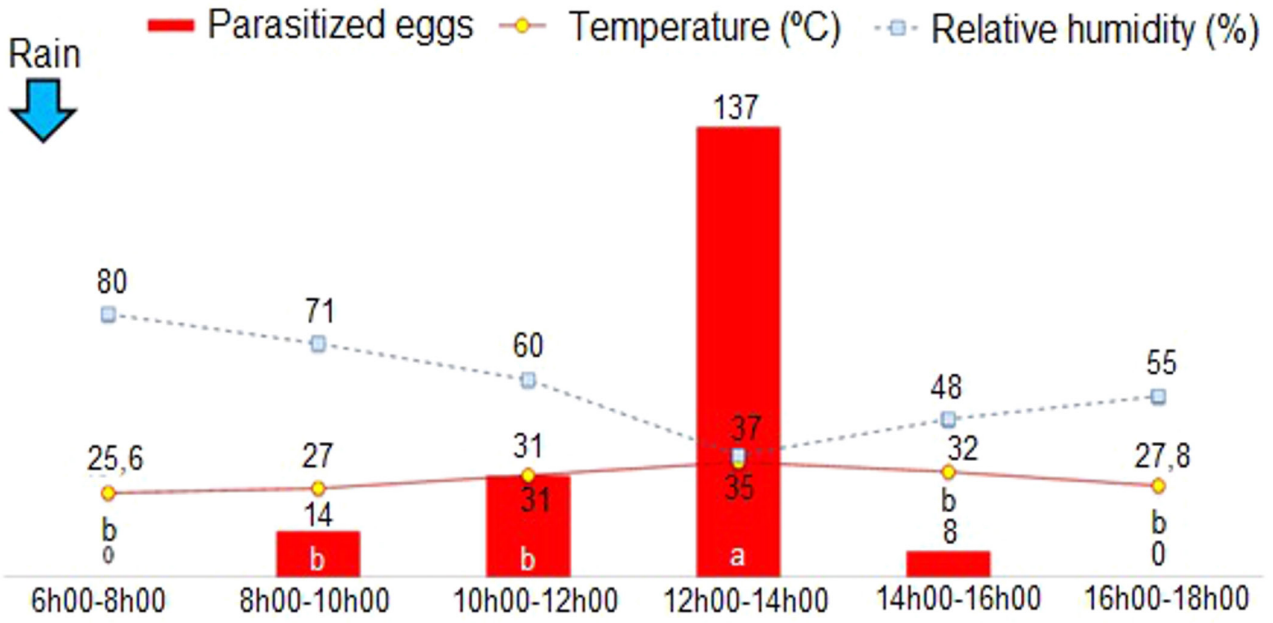
During the evaluation conducted on March 5, 2016, a markedly higher average number of *A. kuehniella* eggs parasitized by *T. pretiosum* was observed between 10:00 and 12:00, exhibiting a significant deviation from both the initial time frame and post-16:00 parasitism levels (Figure 1). During assessment conducted on March 5, 2016, a significant increase in the number of *A. kuehniella* eggs parasitized by T. pretiosum was observed between 10:00 am and 12:00 am, compared to other periods throughout the day. However, statistically significant differences were only observed in relation to assessment times before 8:00 am and after 2:00 pm (Figure 1).

**Figure 1.** Total number of *Anagasta kuehniella* eggs parasitized by *Trichogramma pretiosum* at two-hour intervals in a maize field on March 05, 2013. Columns followed by the same letters do not differ from each other by the Duncan test (p≤0.05).



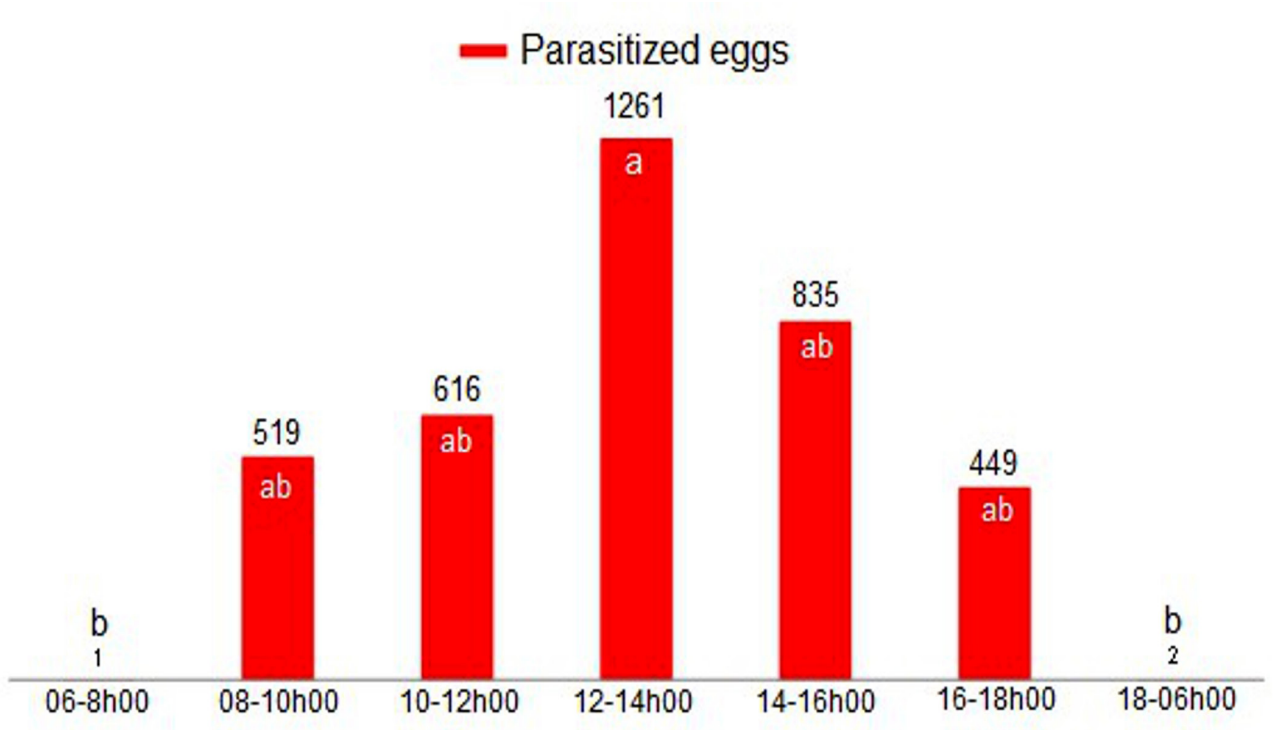

Importantly, an overnight rainfall event led to the loss of all strips containing eggs. Subsequently, on March 15, 2016, the evaluation revealed peak parasitism between 12:00 and 2:00 pm, a pattern distinct from all other intervals (Figure 2). Early morning rainfall from 06:00 pm to 06:00 am compromised the last evaluation period.

**Figure 2.** Total number of *Anagasta kuehniella* eggs parasitized by *Trichogramma pretiosum* at two-hour intervals in a maize field on March 15, 2013. Columns followed by the same letters do not differ from each other by the Duncan test (p≤0.05).




In the evaluation on May 3, 2017, the highest parasitism level was observed from 12:00 to 2:00 pm, differing only from intervals from 6:00 am to 8:00 am and from 6:00 pm to 6:00 am (see Figure 3). Notably, no rainfall occurred on this specific day. The environmental conditions were characterized by elevated temperature and relative humidity, with peak parasitism occurring from noon to 2:00 pm. The amount of parasitized eggs significantly exceeded those in other bioassays, and although relative humidity was elevated, it regrettably remained unmeasured.

**Figure 3.** Total number of *Anagasta kuehniella* eggs parasitized by *Trichogramma pretiosum* at two-hour intervals in a maize field on May 03, 2014. Columns followed by the same letters do not differ from each other by the Duncan test (p≤0.05).



Throughout the day, parasitism rates remained generally consistent, with numerically higher values observed around noon. However, it is imperative to highlight that parasitism consistently remained low from 06:00 am to 08:00 am. Lower parasitism levels were also observed between 6:00 pm and 6:00 am. This phenomenon aligns with findings reported by Pereira et al. (2007), who, through the examination of various parasitoid species in diverse temperature environments, attributed temperature variations to the variance in the parasitism of *Trichogramma* species. For instance, the maximum parasitism rates of *T. pratissolii* on Corcyra and *A. kuehniella* eggs were observed between 24 and 30°C (Zago et al., 2007), *whereas T. exiguum* on *P. xylostella* eggs exhibited the highest parasitism rates at 25°C (Pereira et al., 2007).

**Second parasitoid release bioassay**

During evaluations conducted in 2018, a year characterized by drought and atypical weather conditions, experiments were primarily conducted during periods of high temperature and absence of rainfall.

On February 17, 2018, the highest average number of *A. kuehniella* eggs parasitized by *T. pretiosum* was observed in the afternoon, specifically from 12:00 am to 2:00 pm, followed by the two preceding morning periods (Figure 4). Each of bioassays started at 6:00 am. On March 13, 2018, due to data loss, weather-related information was unavailable. Nevertheless, peak parasitism was observed from 2:00 to 4:00 pm, followed by 10:00 to 12:00 am and 12:00 am to 02:00 pm (Figure 5). In the parasitism peak observed on March 16, 2018 release occurred from 10:00 to 12:00 am, followed by the period from 12:00 am to 2:00 pm (Figure 6). The average temperature exceeded 45ºC during peak parasitism. However, it is important to note that this reading may have been influenced by direct sunlight exposure of the temperature-measuring device.

**Figure 4.** Total number of *Anagasta kuehniella* eggs parasitized by *Trichogramma pretiosum* at two-hour intervals in crotalaria field on February 17, 2015. Columns followed by the same letters do not differ from each other by the Tukey test (p≤0.05).

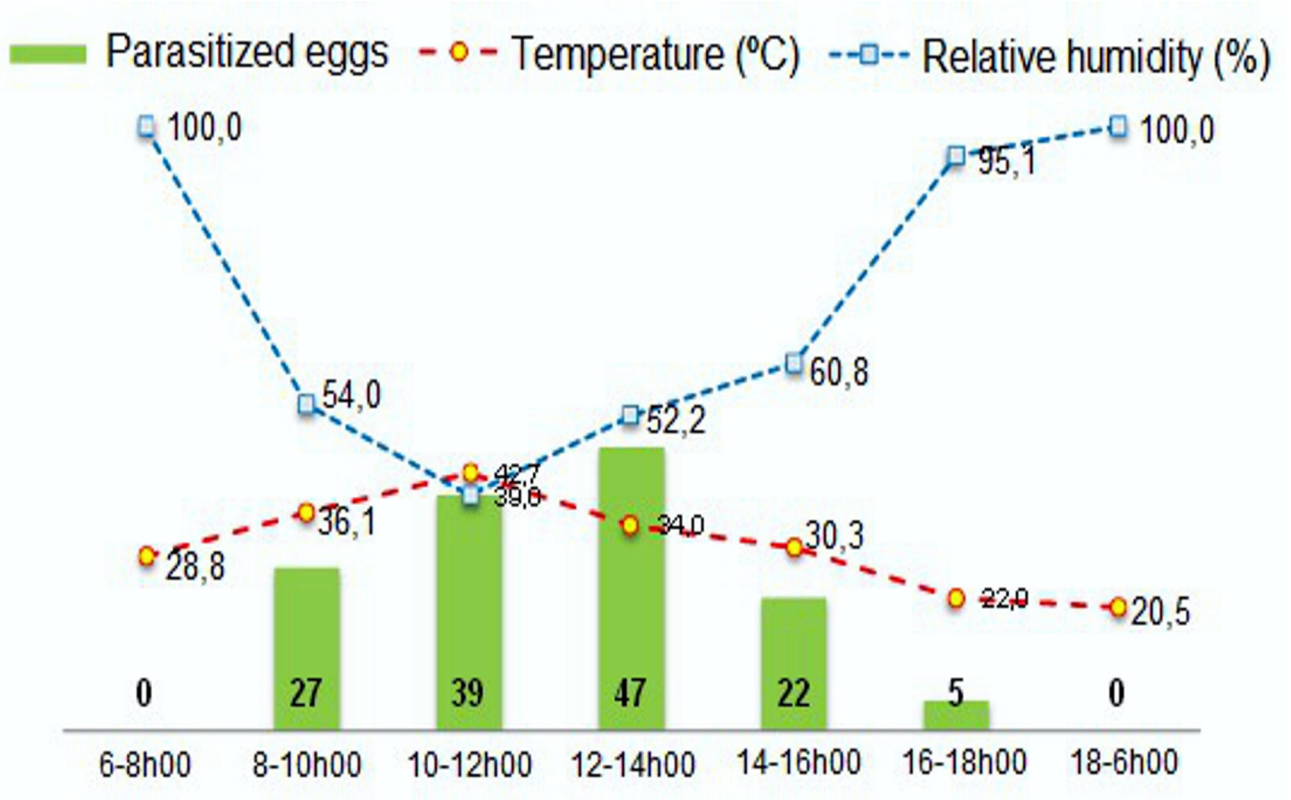


**Figure 5.** Total number of *Anagasta kuehniella* eggs parasitized by *Trichogramma pretiosum* at two-hour intervals in maize field on March 13,2015. Columns followed by the same letters do not differ from each other by the Tukey test (p≤0.05).

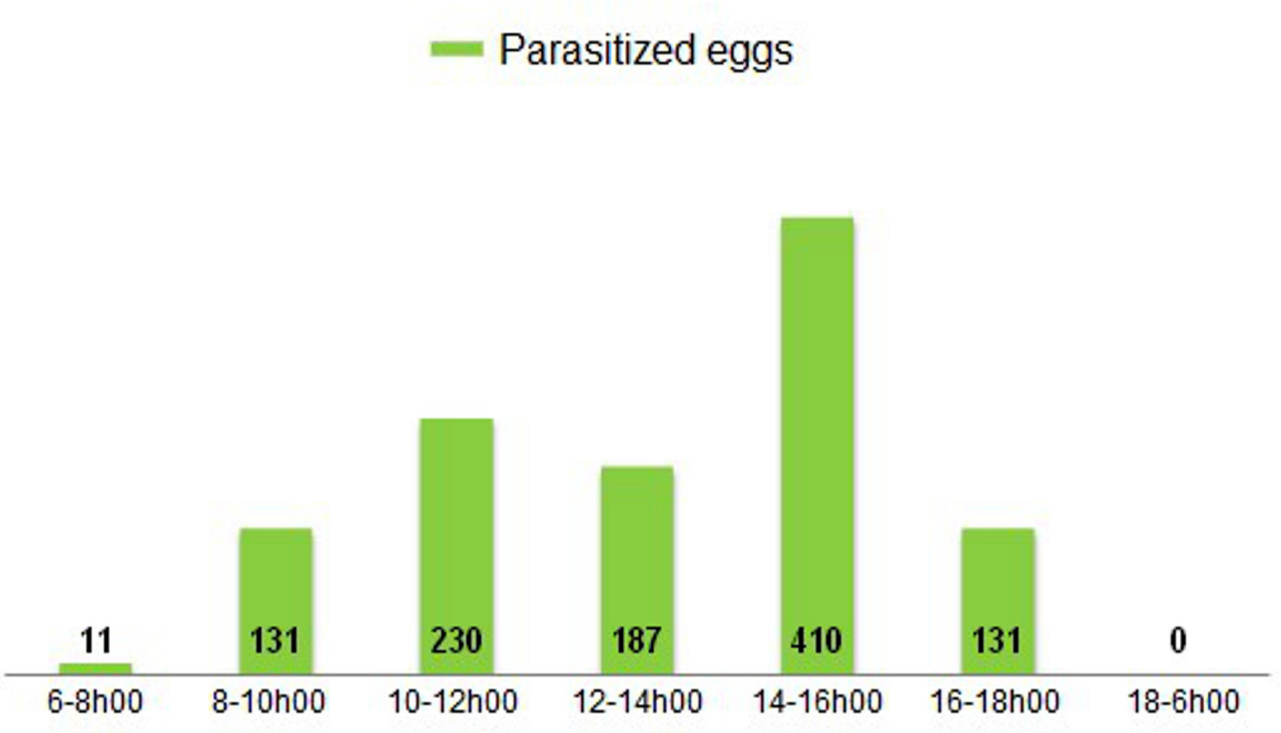
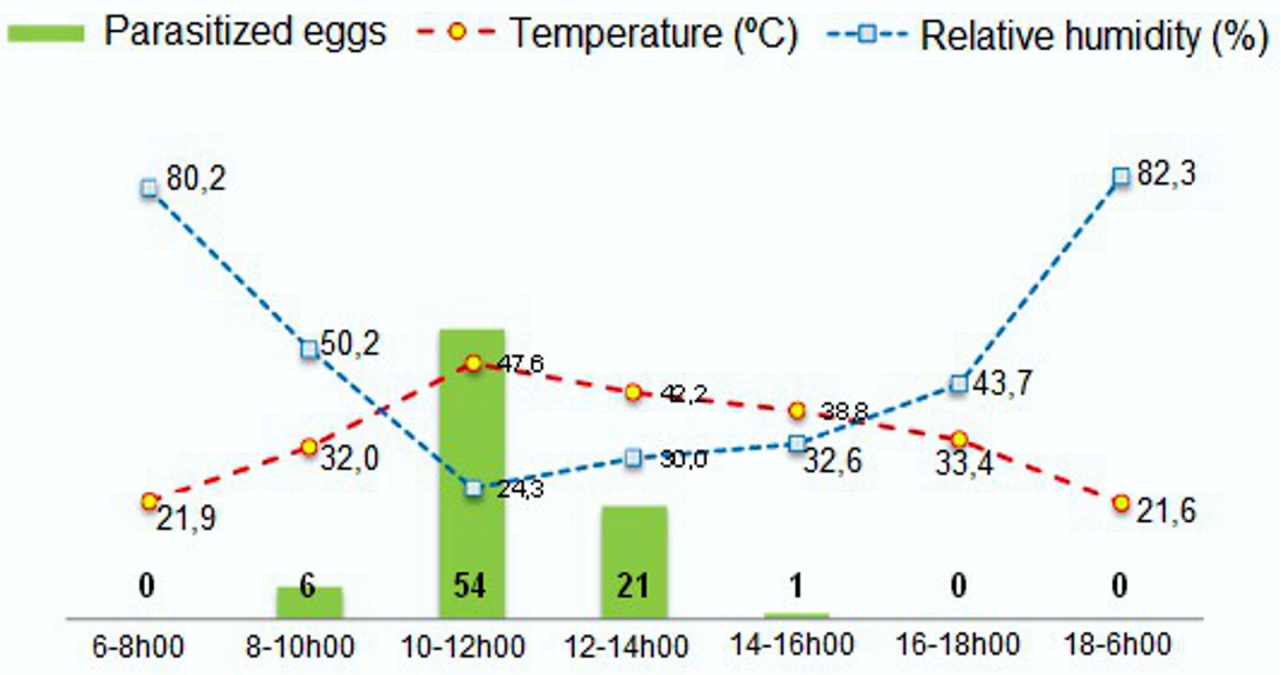



**Figure 6.** Total number of *Anagasta kuehniella* eggs parasitized by *Trichogramma pretiosum* at two-hour intervals in maize field on March 16, 2015. Columns followed by the same letters do not differ from each other by the Tukey test (p≤0.05).




On June 1, 2018, when released in maize, peak parasitism was observed from 12:00 am to 2:00 pm, followed by 10:00 to 12:00 pm (Figure 7). Meanwhile, in sorghum, peak parasitism occurred from 10:00 to 12:00 am, followed by 2:00 to 4:00 pm and 12:00 am to 2:00 pm (refer to Figure 8). During the final release on August 30, 2018, high total number of parasitized eggs was observed, with significant peak from 12:00 am to 2:00 pm (Figure 9).

**Figure 7.** Total number of *Anagasta kuehniella* eggs parasitized by *Trichogramma pretiosum* at two-hour intervals in maize field on June 01, 2015. Columns followed by the same letters do not differ from each other by the Tukey test (p≤0.05).

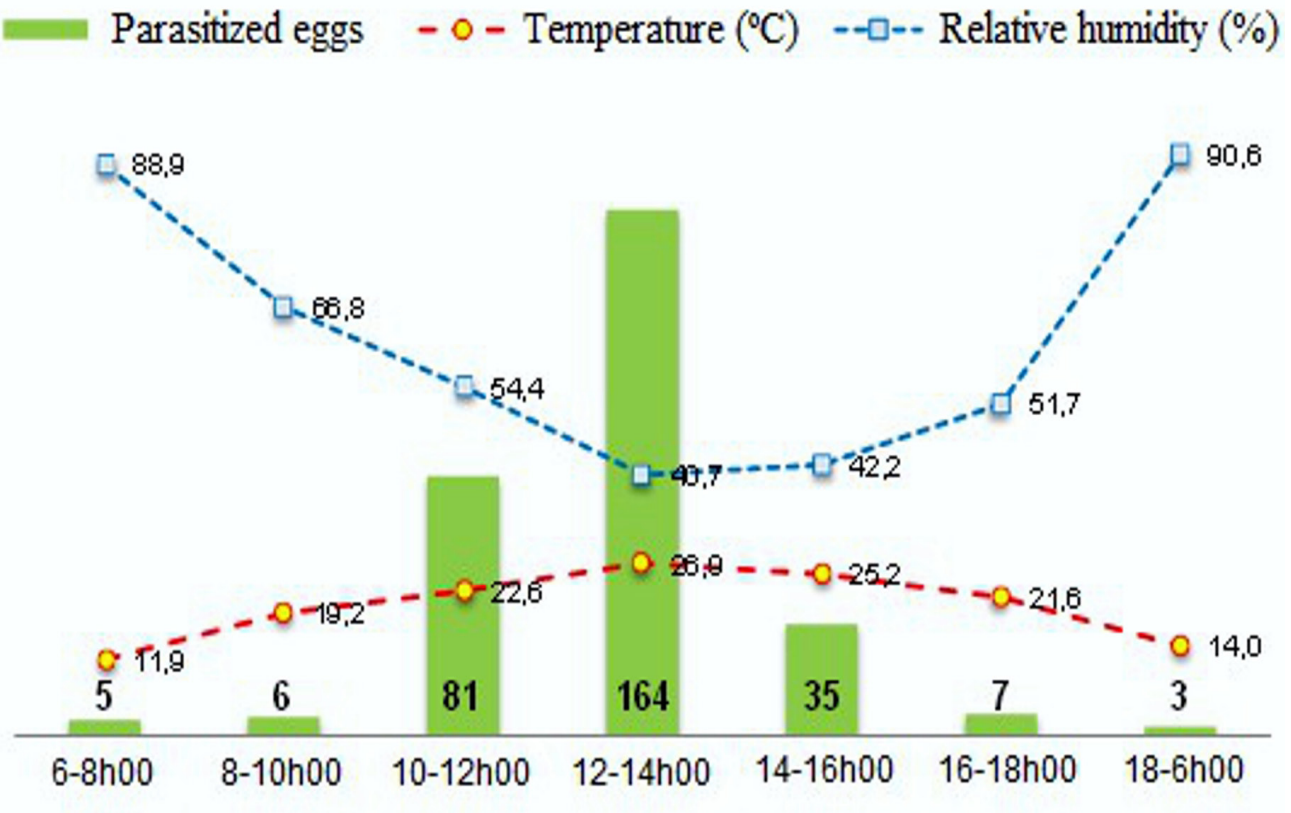



**Figure 8.** Total number of *Anagasta kuehniella* eggs parasitized by *Trichogramma pretiosum* at two-hour intervals in sorghum field on June 01, 2015. Columns followed by the same letters do not differ from each other by the Tukey test (p≤0.05).

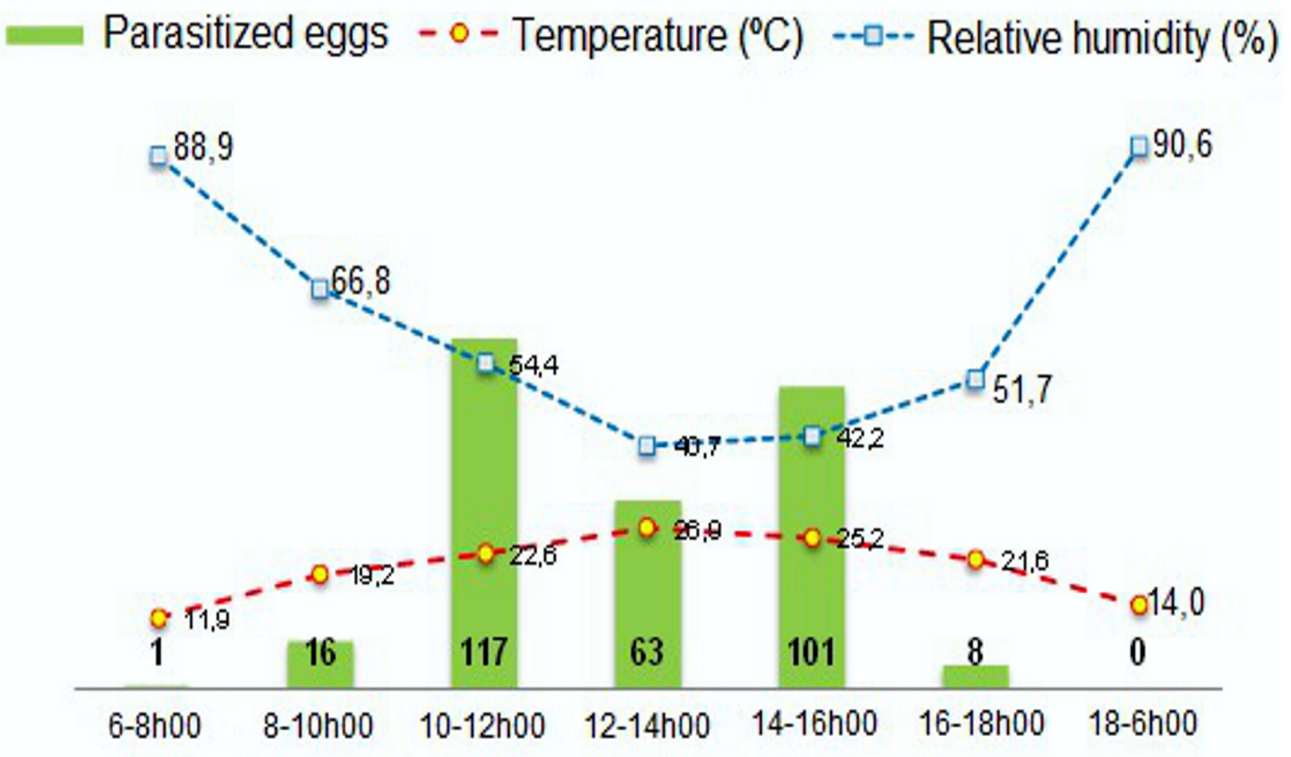
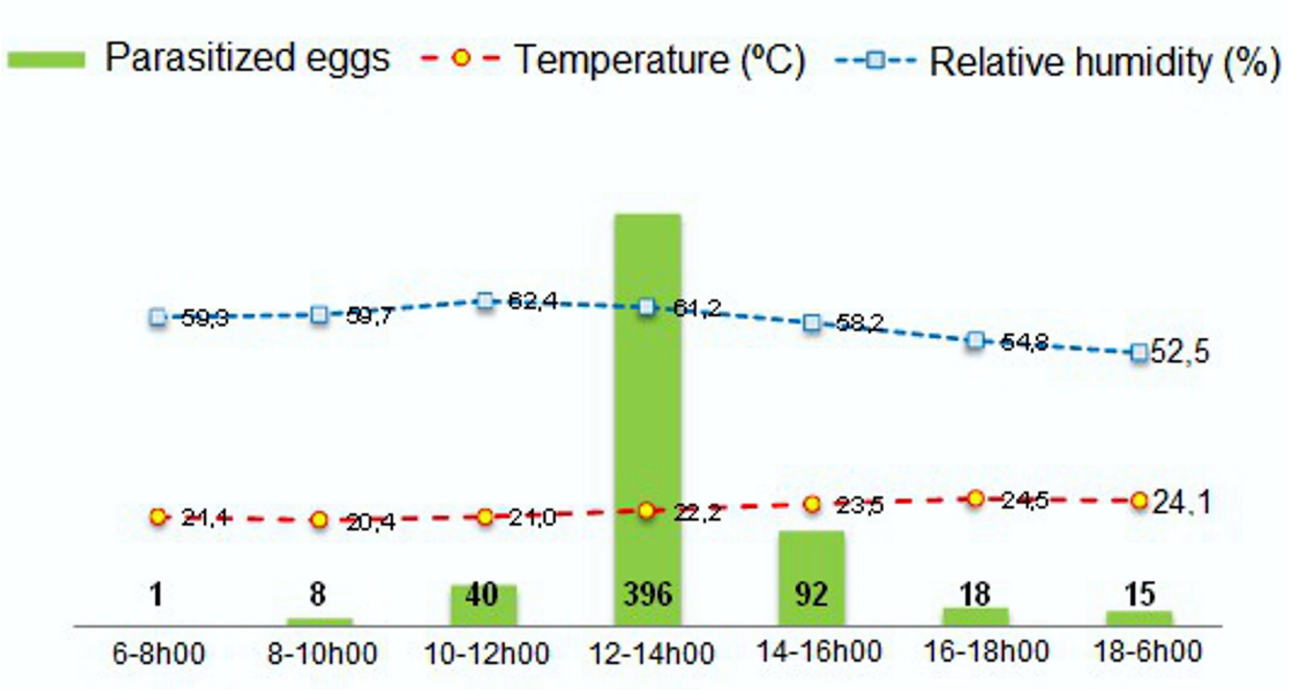


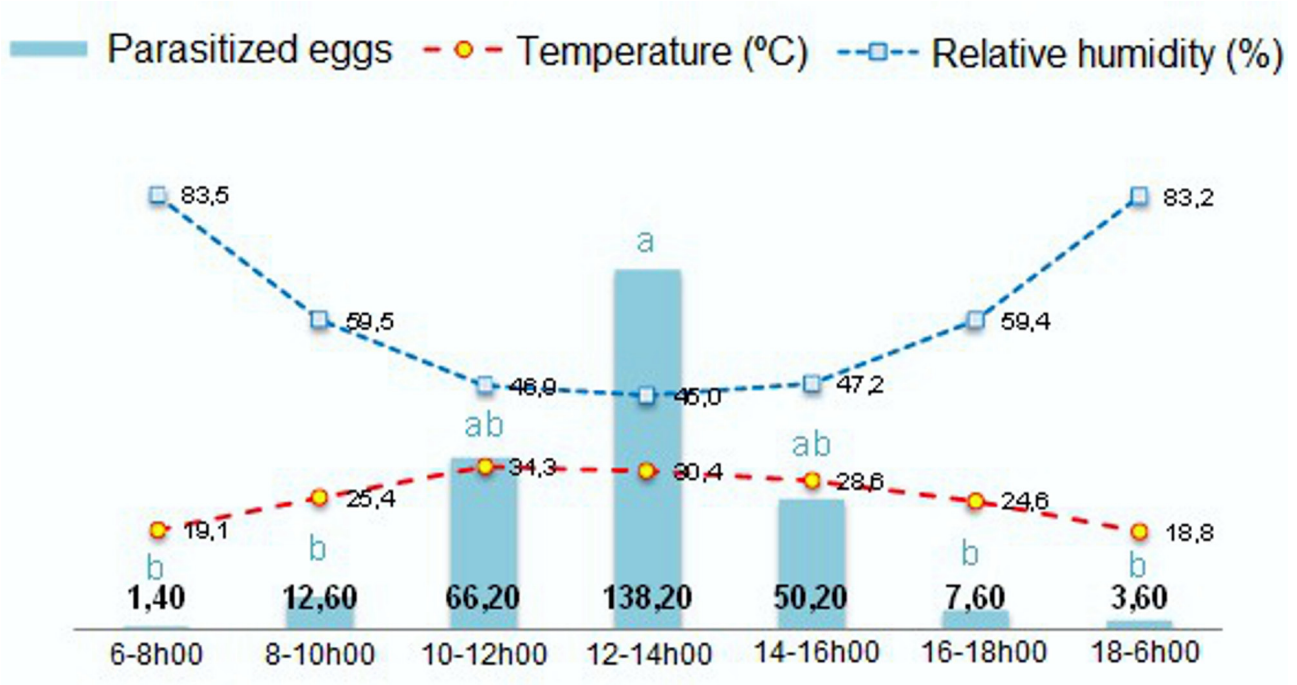
**Figure 9.** Total number of *Anagasta kuehniella* eggs parasitized by *Trichogramma pretiosum* at two-hour intervals in maize field August 30, 2015. Columns followed by the same letters do not differ from each other by the Tukey test (p≤0.05).




Consistently, relative humidity was observed to influence parasitism effectiveness. On release dates with the highest number of parasitized eggs, the relative humidity consistently remained above 40% (Figures 7, 8, and 9).

When averaging all *T. pretiosum* release dates, the highest total average number of parasitized eggs was observed from 12:00 am to 2:00 pm, followed by 10:00 to 12:00 am and 2:00 to 4:00 pm (Figure 10). In general, bioassays demonstrated high average temperatures and high relative humidity.

**Figure 10.** Total average number of *Anagasta kuehniella* eggs parasitized by *Trichogramma pretiosum* at two-hour intervals in the field of the different bioassays on 2015. Columns followed by the same letters do not differ from each other by the Tukey test (p≤0.05).



In conclusion, *T. pretiosum* exhibited peak parasitic activity shortly after being released as adults in the morning on non-rainy days. In the presence of morning rainfall, peak parasitism shifted to the early afternoon. While peak parasitism typically occurred from 10:00 am to 4:00 pm, the peak time in sorghum differed from that in maize.

These findings highlight the importance of further studies to better understand the impact of climatic conditions on the parasitic activity of *Trichogramma* spp. More comprehensive climatological data affecting parasitism can significantly contribute to the development of more successful strategies for these parasitoids.

The present study concludes that the parasitic wasp *T. pretiosum* exhibits higher parasitism rate on *A. kuehniella* eggs during periods of heightened light intensity. Rainfall, on the other hand, negatively impacts parasitism. Furthermore, parasitism is non-existent immediately after the release of wasps and during night-time hours, when temperatures are milder. These results are in line with those described by Carvalho et al. (2014), who evaluated the parasitism capacity of *T. pretiosum* on *Trichoplusia ni* eggs at different temperatures. In contrast to the present study, Cascone et al. (2015) reported enhanced parasitoid attack rates against the tomato borer, *Tuta absoluta*, by *T. achaeae* reared on *T. absoluta* eggs laid on tomato leaves. Positive correlation was observed between temperatures during parasitoid development and fertility at varying temperatures. This information can help inform the optimal timing and environmental conditions for releasing *T. pretiosum* for pest control, as optimal temperatures can greatly fluctuate among species. Further research is suggested to better understand and optimize the influence of climatic conditions on the parasitism behaviour of *Trichogramma* species.

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