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2 **Investigation on the biological characteristics**  
3 **and thermal requirements of egg parasitoid**  
4 ***Trichogrammatoideabactrae* on eggs of cotton**  
5 **Pink bollworm**  
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10 **ABSTRACT**  
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The biological traits and thermal needs of *Trichogrammatoideabactrae* Nagaraja was assessed on the eggs of the Pink bollworm, *Pectinophoragossypiella* to predict the ideal temperature for the release of the parasitoid. A card with 300 *P. gossypiella* eggs was offered to *T. bactrae* adults in a 5:1 ratio at temperatures 18, 20, 22, 24, 26, 28, 30, 32, 34 and 36 °C. All developmental stages of *T. bactrae* were monitored at each temperature, however, no parasitism or development occurred at temperature 36°C. The highest parasitism rate was observed at 28°C (76.03%), and emergence rates exceeded 85.65% between 18 and 30°C. Temperature variations affected both the development duration and adult longevity, with higher temperatures leading to shorter development times and reduced lifespan. The highest proportion of female offspring was recorded at 20°C (73.67%). The total heat requirement for complete development was calculated to be 136.98 degree-days (K), with a lower threshold temperature identified at 11.75°C.

12  
13 *Keywords: Biological control, Cotton, Trichogrammatoideabactrae, thermal requirements,*  
14 *Pink bollworm, Pectinophoragossypiella*

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17 **1. INTRODUCTION**  
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19 Cotton is an important commercial crop in India, accounting for 40% of the global cotton  
20 cultivation area and 21% of global production (AICRP, 2023). Cotton plays an important role  
21 in India's economy, occupies largest acreage and highest production in the world. However,  
22 the crop is being attacked by more than 251 arthropod pests, among them 12 are major  
23 pests which cause significant economic damage to the cotton (Nagrare et al., 2022). Among  
24 the major insect pests of cotton, Pink bollworm, *Pectinophoragossypiella* emerged as a major  
25 pest of cotton with the development of resistance against BG I and BG II affecting cotton  
26 yield and quality (Naik et al., 2018; Nagrare et al., 2022). The overuse of insecticides and the  
27 development of resistance, make it essential to find alternative and environmentally friendly  
28 pest management methods (Asha et al., 2019). Integrated pest management, including the  
29 use of biological methods such as the egg parasitoid *Trichogramma*, is considered a  
30 promising approach. *Trichogramma* species are widely used as biological agents due to their  
31 ease of mass rearing and specificity in targeting pests without harming beneficial insects or  
32 the environment (Molnar et al. 2016). The effectiveness of *Trichogramma* species depends  
33 on various factors, including species potential, host interaction, thermal requirement, and  
34 climatic conditions (Bourchier and Smith 1996; Hassan 1994). The study of thermal  
35 requirements facilitates the understanding of the relationship between temperature and  
36 biological control agents (Reznik and Voinovich 2015). The laboratory studies of  
37 *Trichogramma* spp. can provide a basis to predict the optimum temperature required for

38 maximum percent parasitisation, percent emergence, developmental duration, adult survival,  
39 and the percent female progeny at known temperatures. The study aims to evaluate the  
40 biological parameters and thermal requirements of *Trichogrammatoideabactrae* on the host  
41 cotton pink bollworm at different temperatures.

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## 43 2. MATERIAL AND METHODS

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45 The experiment was conducted at the insect biocontrol laboratory, ICAR-Central Institute of  
46 Cotton Research (CICR) Nagpur, during 2023-24. The study involved the mass multiplication  
47 of egg parasitoid *T. bactrae*, a parasitoid wasp and the rearing of the host insect, *P.*  
48 *gossypiella*.

49

50 *T. bactrae* was reared on the eggs of the factitious host, the rice moth (*Corcyra*  
51 *cephalonica*). The eggs of *C. cephalonica* were collected from the mass rearing facility, and  
52 were cleaned and sterilized with UV light to prevent hatching. These sterilized eggs were  
53 then used to prepare Trichocards, which were attached to nucleus cards containing  
54 parasitized eggs and placed inside a polythene bag for parasitization. After 3 to 4 days, the  
55 parasitized eggs in the Trichocards turned black, indicating successful parasitization. The  
56 Trichocards were then labelled and stored in a refrigerator for further multiplication.

57

58 The *P. gossypiella* population was reared on an artificial diet as described by Naik et  
59 al. (2017). For all the experiment, one-day-old *P. gossypiella* eggs were used. Approximately  
60 300 eggs were glued onto paper cards and presented to *T. bactrae* adults at a 5:1 ratio, with  
61 three replications conducted at temperatures of 18, 20, 22, 24, 26, 28, 30, 32, 34 and 36 °C.  
62 Biological parameters, including development period, parasitization rate, emergence rate,  
63 longevity, percentage of female progeny, as well as thermal requirements such as the lower  
64 thermal threshold and thermal constant, were evaluated for *T. bactrae* on cotton pink  
65 bollworm.

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### 67 2.1 Statistical analysis:

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69 The experimental data were subjected to statistical analysis using a completely randomized  
70 design (CRD) with the help of R software (R core team 2021). Prior to the analysis, normality  
71 of the data was checked, and appropriately transformed using arcsine and square root  
72 transformations if required. The treatment means were separated using the Duncan's  
73 multiple range test (DMRT) ( $p=0.05$ ).

74

75 The following formulas were used for the calculation of the various biological parameters.

76

$$\text{Percent Parasitisation} = \frac{\text{No. of PBW eggs changed to black colour}}{\text{Total PBW eggs kept for parasitisation}} \times 100$$

77

$$\text{Percent emergence} = \frac{\text{No. of parasitised PBW eggs with emergence holes}}{\text{Total No. of parasitised eggs}} \times 100$$

78

$$\text{Percent female progeny} = \frac{\text{number of females}}{\text{number of females} + \text{number of males}} \times 100$$

79

80 The development period (Egg-adult) was calculated by counting the number of days the  
81 adult lived till the mortality from the day just after parasitisation. Adult longevity was  
82 determined by counting the number of days required by an adult to complete the life cycle  
from emergence to death of the adult at different constant temperatures in BOD.

83

84 In addition to this, the calculation of lower threshold temperature and thermal  
85 constant in degree days were then determined by regressing the development rate on  
86 temperature (Kipyatkov and Lopatina 2010).

87

88 Thermal constant (K) = Development duration (Temperature - Development threshold)

89

$$K = D (T - T_0)$$

90

91 Where, T = temperature at which insect species is reared, K = reciprocal of the regression  
92 coefficient (b) computed between development rate and temperature

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94 **Obtaining the equation:**  $Y = a + bx$

95

96 By definition, the insect stops developing at the lower developmental threshold ( $Y = 0$ ):

97 The K was estimated as the reciprocal of the regression coefficient (b) between development  
98 rate and temperature.

99

$$K = 1/b$$

100

101  $T_0$  was determined ratio regression intercept (a) and (b).

102

$$T_0 = - a/b$$

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### 104 3. RESULTS AND DISCUSSION

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106 At a temperature of 36°C *T. bactrae* were incapable of parasitising and development and,  
107 therefore not considered while computing the results.

108

#### 109 3.1 Percent Parasitisation

110 The parasitisation potential of *T. bactrae* against the eggs of *P. gossypiella* at different  
111 temperatures revealed that maximum parasitisation at 28°C (76.03%), followed by 26°C  
112 (74.74%) with minimum parasitisation was recorded at extreme temperature ranges at 34°C  
113 (26.44%) and 18°C (33.54%) (Fig.1). These findings align with those of Jidung et al. (2022),  
114 who reported parasitism rates of 82.71% and 84.71% at 25°C when *Trichogramma chilonis*  
115 was reared on eggs of *Corcyra cephalonica* and *Plutella xylostella*, respectively. The results  
116 obtained were similar to the findings of Malik (2001) reported that maximum parasitisation of  
117 95.81% was found at a temperature of 28°C when *T. bactrae* was reared on *P. gossypiella*  
118 eggs.

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#### 120 3.2 Percent adult Emergence

121 The data indicated that there is a significant difference in percent emergence of *T. bactrae* at  
122 different temperature treatments. However, exhibiting the highest percent emergence at  
123 26°C (98.81%). The next most effective treatment occurred at 20°C (98.41%), while the  
124 lowest percent emergence was recorded at 34°C (75.56%) (Fig.1). The highest percentage  
125 of adult emergence, consistent with Asha et al. (2020), who found maximum adult  
126 emergence (95.82%) at 25°C, and 92.22% at 28°C when *T. bactrae* was reared on *P.*  
127 *gossypiella* eggs. The findings supported previous research by Coutinho et al. (2021), which  
128 indicated that the highest rate of adult emergence was at 25°C (95.82%) in both the strains  
129 of *T. preteosum* reared on eggs of *Neoleucinodes elegantalis* at different temperatures.

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#### 131 3.3 Developmental period (Egg to adult) of *Trichogramma* species on eggs of *P.* 132 *gossypiella*

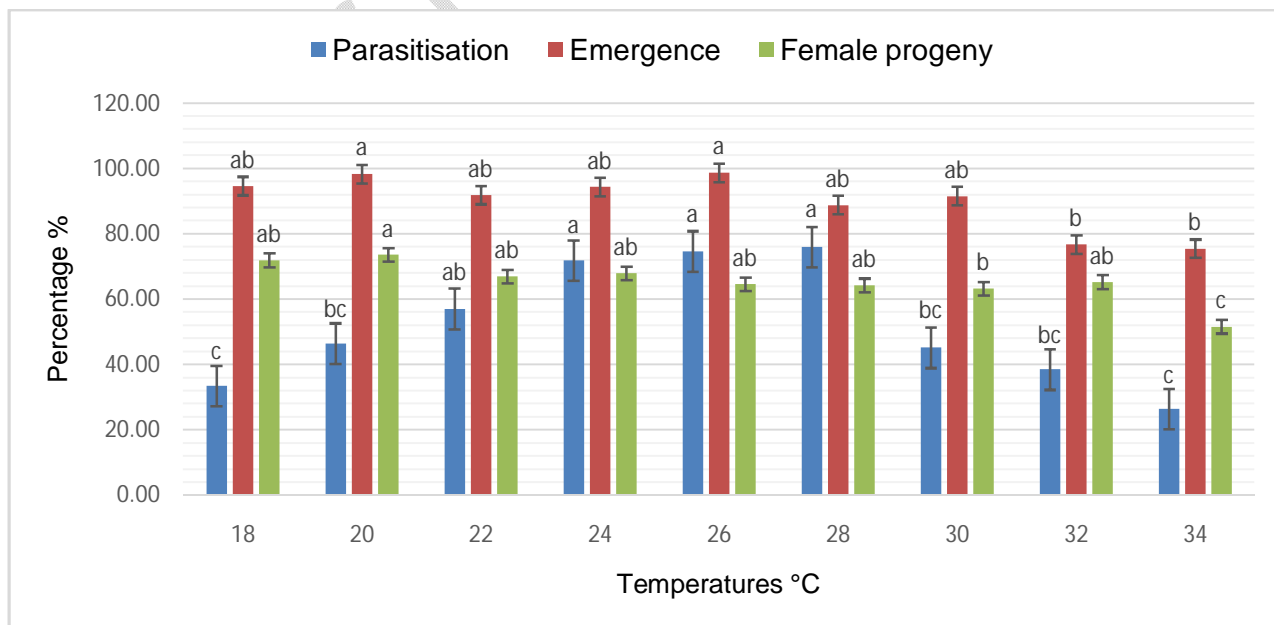
133 The longest development period recorded was 23.33 days at a temperature of 18°C,  
 134 followed by 19.33 days at 20°C and 13.67 days at 22°C. Conversely, the shortest  
 135 development period was noted at 34°C (Fig.1). It was observed that with the temperature  
 136 increased, the development period decreased up to a threshold of 34°C, beyond which no  
 137 development occurred at 36°C. These results are in agreement with Hutchison et al. (1990),  
 138 who reported that the development period of *T. bactrae* decreased from 22.8 days at 15°C to  
 139 7.1 days at 32.5°C for males, and from 28.4 days at 15°C to 7.3 days at 32.5°C for  
 140 females. The present findings are in line with Pino et al. (2020), concluded that development  
 141 time decreases with an increase in temperature from 15 °C, 20°C, 25°C, and 30°C in  
 142 parasitoid species of *Trichogramma chacaee* reared on the eggs of *Ephestiakuehniella*.  
 143

### 144 3.4 Adult longevity

145 The maximum recorded lifespan for adult *T. bactrae* is 9.33 days at a temperature of 18°C,  
 146 which is also matched at 20°C. In contrast, the minimum lifespan observed is 2.33 days at  
 147 34°C. Therefore, as the temperature increases from 18°C to 34°C, the longevity of adults  
 148 decreases from 9.33 days to 2.33 days, respectively (& Fig. 2). Similarly, Tabeboardbar et  
 149 al. (2022) observed that temperature significantly influenced the longevity of female progeny,  
 150 showing an inverse relationship, with longevity decreasing from 12.74 days at 22.5°C to 5.74  
 151 days at 40°C when *T. euproctidis* was reared on *Ephestiakuehniella* eggs. Similar results  
 152 were shown by Atashi et al. (2023) when *T. euproctidis* females reared on the eggs of *H.*  
 153 *armigera* at different constant temperatures with 11.60 days to 4.57 days at 21 °C to 33°C  
 154 respectively for females and 9.30 days to 3.03 days at 21°C to 33°C for males respectively.  
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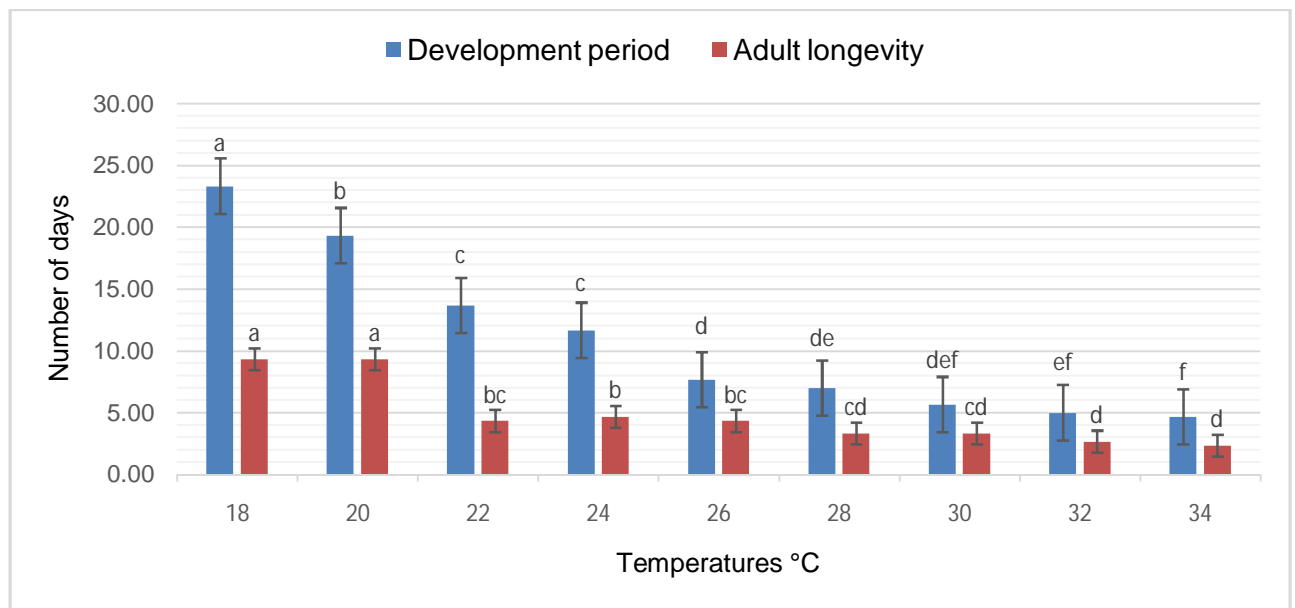
### 156 3.5 Percent female progeny

157 The maximum percent female progeny was observed at 20°C (73.67%), the next best  
 158 treatment at 18°C (72.00%) and 24°C (68%) with a minimum percent female progeny at a  
 159 temperature of 34°C (51.67%). The results also indicated that the percentage of female  
 160 progeny varied with temperature. Similar observations were made by Hutchison et al.  
 161 (1990), who reported that the proportion of female progeny decreased from 77.5% at 15°C to  
 162 51.6% at 30°C. In contrast, Maceda et al. (2003) noted that progeny sex ratios varied  
 163 depending on the parasitoid species and temperature.  
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**fig.1** Percent parasitization, percent emergence, percent female progeny of *T. bactrae* reared on eggs of *P. gossypiella* at different temperatures.



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**Fig. 2** Development period (Egg to adult), and adult longevity of *T. bactrae* reared on eggs of *P. gossypiella* at different temperatures.

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The developmental period of the egg parasitoid *T. bactrae*, measured in days from egg to adult, was utilized as a biological parameter to establish the threshold temperature ( $T_b$ ) and the thermal constant in degree days (K) tested at a different constant temperature (18, 20, 22, 24, 26, 28, 30, 32, 34 and 36°C) on the eggs of *P. gossypiella*. The development of the parasitoid *T. bactrae* began when the temperature remained above the lower threshold temperature of 11.75°C, and the accumulated heat necessary for complete development was 136.98 degree-days(K). The regression equation between temperature and development rate was found as,  $Y = 0.0073x - 0.0858$  ( $R^2 = 0.88$ ). The thermal requirements observed in this study were consistent with those reported by Hutchison et al. (1990), who found that *T. bactrae* required a lower developmental threshold of 9.54°C and 143.16 degree-days for complete development. However, Carvalho et al. (2017) reported that *Trichogrammapretiosum* TM exhibits a lower threshold temperature of 10.82°C and the cumulative heat required for complete development was 135.55 degree-days(K) when reared on the eggs of *H. armigera*.

#### 4. CONCLUSION

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The study revealed the optimal temperature range for both parasitism and adult emergence for *T. bactrae* was found to be between 24°C and 28°C. This temperature range would be the ideal for the release of the parasitoid to get the maximum parasitism benefit. The development period and adult longevity were longest at 18°C and shortest at 34°C. A temperature of 20°C yielded a higher percentage of female progeny, while 26°C resulted in the maximum number of progeny per host. The lower threshold temperature ( $T_0$ ) and thermal constant (K) were determined to be 11.75°C and 136.98 degree-days, respectively.

199 The analysis clearly showed that temperature has a significant influence on the life cycle of  
200 this egg parasitoid.

201

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203

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207

## 208 **COMPETING INTERESTS**

209

210 Authors have declared that no competing interests exist

211

## 212 **AUTHORS' CONTRIBUTIONS**

213

214 Sahil D. Meshram and PrabhulingaTenguridesigned the study,performed the statistical  
215 analysis, wrote the protocol, andwrote the first draft of the manuscript. V. K. Biradar<sup>1</sup>, Shivaji  
216 H. Thube, Babasaheb B. Fand, Madhu T. N., Vivek Shah, Rachna Pande and V. Chinna  
217 Babu Naik managed the analyses ofthe study and the literature searches. All authors read  
218 and approved the final manuscript

219

## 220 **Disclaimer (Artificial intelligence)**

221 Author(s) hereby declare that NO generative AI technologies such as Large Language  
222 Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the  
223 writing or editing of this manuscript.

224

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