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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.

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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). However, the pink bollworm,
21 *Pectinophoragossypiella* is a major pest that affects cotton yield and quality. The overuse of
22 insecticides has led to the development of resistance, making it essential to find alternative
23 and environmentally friendly methods of pest management (Asha, 2019). Integrated pest
24 management, including the use of biological methods such as the egg parasitoid
25 *Trichogramma*, is considered a promising approach. *Trichogramma* species are widely used
26 as biocontrol agents due to their ease of mass rearing and specificity in targeting pests
27 without harming beneficial insects or the environment (Molnar et. al 2016). The
28 effectiveness of *Trichogramma* species depends on various factors, including species
29 potential, host interaction, and climatic conditions (Bourchier and Smith 1996; Hassan 1994).
30 Thus, the study aims to evaluate the biological parameters and thermal requirements of
31 *Trichogrammatoideabactrae* on the host cotton pink bollworm at different temperatures to
32 predict the ideal temperature for the release of the parasitoid for management of cotton pink
33 bollworm.
34

35 **2. MATERIAL AND METHODS**

36

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

41

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

49

50 The *P. gossypiella* population was reared on an artificial diet as described by Naik et
51 al. (2017). For all the experiment, one-day-old *P. gossypiella* eggs were used. Approximately
52 300 eggs were glued onto paper cards and presented to *T. bactrae* adults at a 5:1 ratio, with
53 three replications conducted at temperatures of 18, 20, 22, 24, 26, 28, 30, 32, 34 and 36 °C.
54 Biological parameters, including development period, parasitization rate, emergence rate,
55 longevity, percentage of female progeny, as well as thermal requirements such as the lower
56 thermal threshold and thermal constant, were evaluated for *T. bactrae* on cotton pink
57 bollworm. The following formulas were used for calculation of the various biological
58 parameters.

59

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

60

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

61

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

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

66

67 The calculation of lower threshold temperature and thermal constant in degree days were
68 then determined by regressing development rate on temperature (Kipyatkov and Lopatina
69 2010).

70

71 Thermal constant (K) = Development duration (Temperature - Development threshold)
72
$$K = D (T - T_0)$$

73

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

76

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

78

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

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

$$82 \quad K = 1/b$$

83

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

85

$$86 \quad T_0 = - a/b$$

87 **3. RESULTS AND DISCUSSION**

88

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

91

92 **3.1 Percent Parasitisation**

93 The parasitisation potential of *T. bactrae* against the eggs of *P. gossypiella* at different
94 temperatures revealed that maximum parasitisation at 28°C (76.03%), followed by 26°C
95 (74.74%) with minimum parasitisation was recorded at extreme temperature ranges at 34°C
96 (26.44%) and 18°C (33.54%) (Fig.1). These findings align with those of Jidung et al. (2022),
97 who reported parasitism rates of 82.71% and 84.71% at 25°C when *Trichogramma achilonis*
98 was reared on eggs of *Corcyra cephalonica* and *Plutellaxylostella*, respectively. However,
99 our results differ from Malik (2001), who found a parasitism rate of 95.81% at 28°C when *T.*
100 *bactrae* was reared on *Pectinophoragossypiella* eggs.

101

102 **3.2 Percent adult Emergence**

103 The data indicated that there is a significant difference in percent emergence of *T. bactrae* at
104 different temperature treatments. However, exhibiting the highest percent emergence at
105 26°C (98.81%). The next most effective treatment occurred at 20°C (98.41%), while the
106 lowest percent emergence was recorded at 34°C (75.56%) (Fig.1). The highest percentage
107 of adult emergence, consistent with Asha et al. (2020), who found maximum adult
108 emergence (95.82%) at 25°C, and 92.22% at 28°C when *T. bactrae* was reared on *P.*
109 *gossypiella* eggs.

110 **3.3 Developmental period (Egg to adult) of *Trichogramma* species on eggs of *P.*** 111 ***gossypiella***

112 The longest development period recorded was 23.33 days at a temperature of 18°C,
113 followed by 19.33 days at 20°C and 13.67 days at 22°C. Conversely, the shortest
114 development period was noted at 34°C (Fig.1). It was observed that with the temperature
115 increased, the development period decreased up to a threshold of 34°C, beyond which no
116 development occurred at 36°C. These results are in agreement with Hutchison et al. (1990),
117 who reported that the development period of *T. bactrae* decreased from 22.8 days at 15°C to
118 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 females.

119 **3.4 Adult longevity**

120

121 The maximum recorded lifespan for adult *T. bactrae* is 9.33 days at a temperature of 18°C,
122 which is also matched at 20°C. In contrast, the minimum lifespan observed is 2.33 days at
123 34°C. Therefore, as the temperature increases from 18°C to 34°C, the longevity of adults
124 decreases from 9.33 days to 2.33 days, respectively (& Fig. 2). Similarly, Tabeboardbar
125 et al. (2022) observed that temperature significantly influenced the longevity of female progeny,

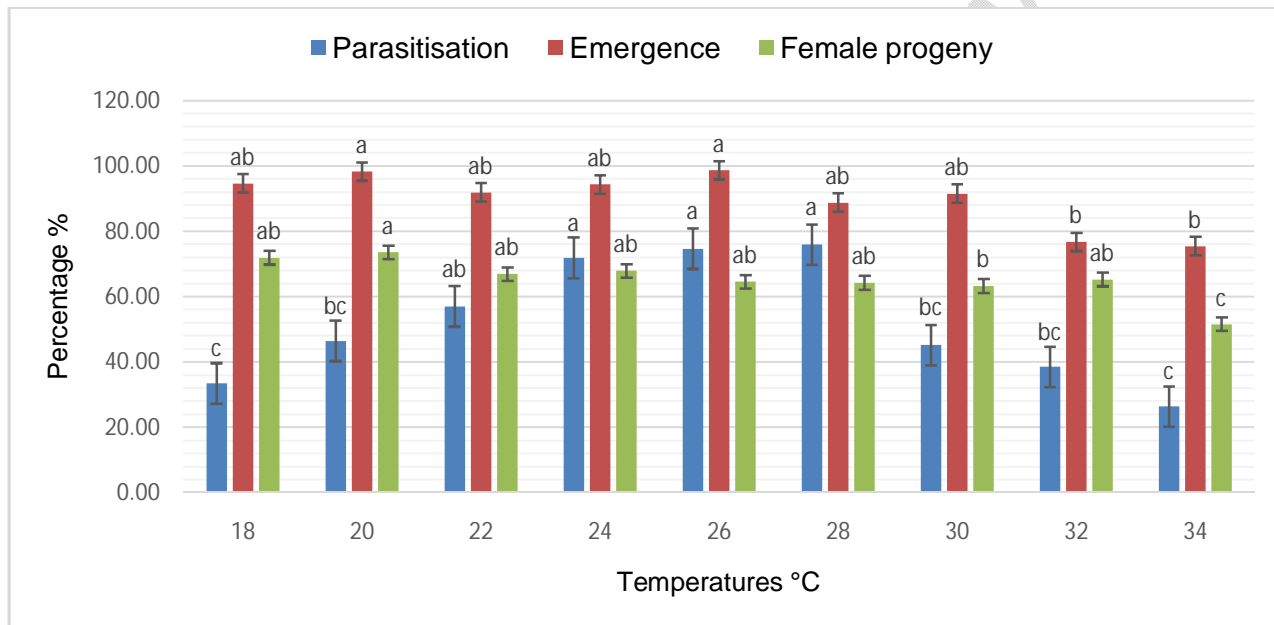
126 showing an inverse relationship, with longevity decreasing from 12.74 days at 22.5°C to 5.74
127 days at 40°C when *T. euproctidis* was reared on *Ephestiakuehniella* eggs.

128

129 3.5 Percent female progeny

130 The maximum percent female progeny was observed at 20°C (73.67%), the next best
131 treatment at 18°C (72.00%) and 24°C (68%) with a minimum percent female progeny at a
132 temperature of 34°C (51.67%). The results also indicated that the percentage of female
133 progeny varied with temperature. Similar observations were made by Hutchison et al.
134 (1990), who reported that the proportion of female progeny decreased from 77.5% at 15°C to
135 51.6% at 30°C. In contrast, Maceda et al. (2003) noted that progeny sex ratios varied
136 depending on the parasitoid species and temperature.

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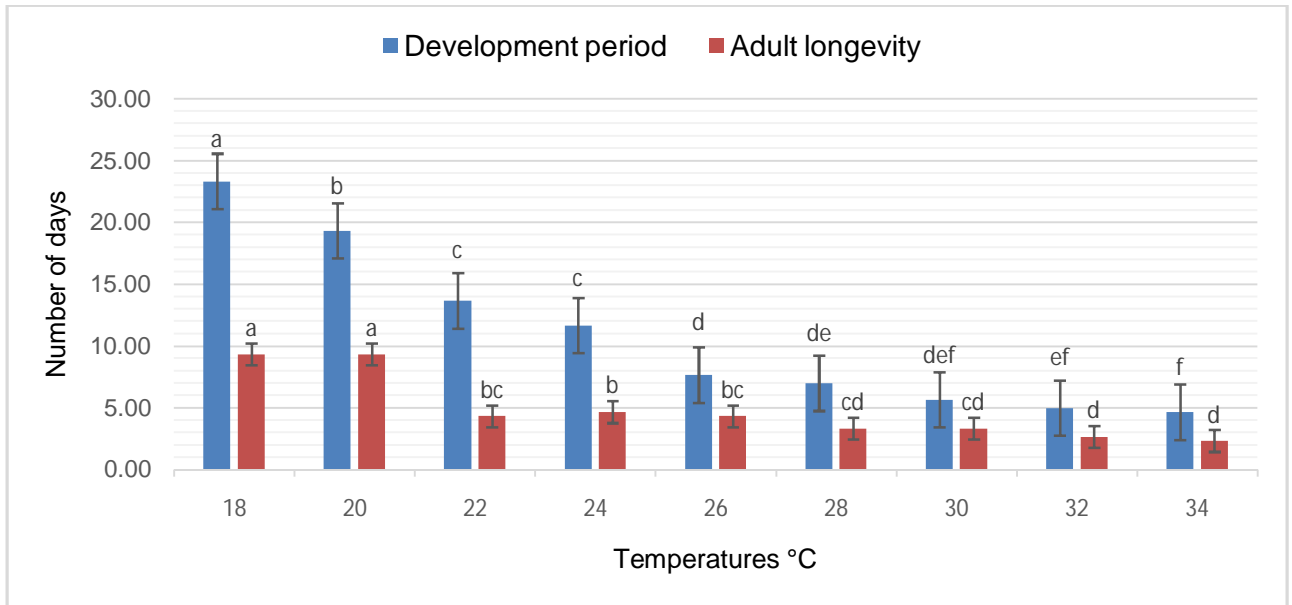
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Fig.1 Percent Parasitisation, Percent Emergence, Percent Female Progeny of *T. bactrae* reared on eggs of *P. gossypiella* at different temperatures.



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

147

148 The developmental period of the egg parasitoid *T. bactrae*, measured in days from egg to
149 adult, was utilized as a biological parameter to establish the threshold temperature (T_b) and
150 the thermal constant in degree days (K) tested at a different constant temperature (18, 20,
151 22, 24, 26, 28, 30, 32, 34 and 36°C) on the eggs of *P. gossypiella*. The development of the
152 parasitoid *T. bactrae* began when the temperature remained above the lower threshold
153 temperature of 11.75°C, and the accumulated heat necessary for complete development
154 was 136.98 degree-days(K). The regression equation between temperature and
155 development rate was found as, $Y = 0.0073x - 0.0858$ ($R^2 = 0.88$). The thermal requirements
156 observed in this study were consistent with those reported by Hutchison et al. (1990), who
157 found that *T. bactrae* required a lower developmental threshold of 9.54°C and 143.16
158 degree-days for complete development

159

160 **4. CONCLUSION**

161

162 The optimal temperature range for both parasitism and adult emergence was found to be
163 between 24°C and 28°C. The development period and adult longevity were longest at 18°C
164 and shortest at 34°C, with the minimum development period for *T. bactrae* recorded at 4.67
165 days. A temperature of 20°C yielded a higher percentage of female progeny, while 26°C
166 resulted in the maximum number of progeny per host. The lower threshold temperature (T_0)
167 and thermal constant (K) were determined to be 11.75°C and 136.98 degree-days,
168 respectively. The analysis clearly showed that temperature has a significant influence on the
169 life cycle of this egg parasitoid.

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171 **ACKNOWLEDGEMENTS**

172

173 The authors are grateful to the Director, ICAR-Central Institute for Cotton Research (CICR)
174 Nagpur, for providing the research facilities and the College of Agriculture, Nagpur for
175 allowing to conduct the research work at CICR Nagpur.

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177 **COMPETING INTERESTS**

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179 Authors have declared that no competing interests exist

180

181 **AUTHORS' CONTRIBUTIONS**

182

183 Sahil D. Meshram and PrabhulingaTenguridesigned the study,performed the statistical
184 analysis, wrote the protocol, andwrote the first draft of the manuscript. V. K. Biradar¹, Shivaji
185 H. Thube, Babasaheb B. Fand, Madhu T. N., Vivek Shah, Rachna Pande and V. Chinna
186 Babu Naik managed the analyses ofthe study and the literature searches. All authors read
187 and approved the final manuscript

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