# Rearing thermal conditions modulate the feeding attributes of *Zygogramma bicolorata* Pallister (Coleoptera, Chrysomelidae)

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**ABSTRACT:** Effects of temperature on the different parameters of consumption and utilization of food, such as consumption index, conversion of ingested food, absolute digestibility, conversion of digested food and growth rate were investigated by rearing *Zygogramma bicolorata* Pallister at 15°C, 20°C, 25°C, 30°C and 35°C. Death of different life stages, including under-developed adults occurred at 15 and 35°C. Maximum consumption index during the feeding period was observed at 25°C, whereas it was minimum at 30°C. The results revealed that conversion of ingested food was maximum at 20°C and minimum at 25°C. In addition, the conversion of digested food was maximum at 20°C and minimum at 25°C. However, absolute digestibility and relative growth rate increased with increasing temperature from 20 to 30°C. © 2024 Association for Advancement of Entomology

KEY WORDS: Temperature, life stages, consumption index, conversion efficiency, growth

### INTRODUCTION

Like all organisms, insects require an energy balance crucial for their growth, development, reproduction, and survival, depending on the equilibrium between energy acquisition and expenditure in physiological processes (Klepsatel *et al.*, 2019). Energy stored as food reserves determine insect's survival in adverse conditions (Rion and Kawecki, 2007). Insects serves as vital energy transformer, as they are integral components of ecosystems. In terrestrial ecosystems, insect herbivores significantly influence plant biomass (Carson and Root, 2000), species diversity (Bagchi *et al.*, 2014), competition dynamics (Kim *et al.*, 2013), and nutrient cycling (Metcalf *et al.*, 2014). As insects are ectothermic, their physiological processes are directly tied to environmental temperature (Fields, 2001). In the last century, Earth's average temperature increased by 1°C and is expected to rise by 0.2°C per decade (Marshall et al., 2020). Studies indicate that temperature significantly influences various aspects of insect such as dispersal, foraging, species interaction (Afaq, 2012; Soga and Gaston, 2018), courtship signaling, mating frequency, species recognition (Larson et al., 2019), movement, recolonization (Fletcher et al., 2018), development, predation, herbivory (McMunn et al., 2019; Owens et al., 2020), initiation, and termination of diapause (Dalin et al., 2010; Tougeron et al., 2020), as well as population growth rate (Miles et al., 2019; Murphy et al., 2020). Additionally, temperature

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plays a role in modulating chemically mediated signals, phenology, life history (Gallinat *et al.*, 2015; Ekholm *et al.*, 2019; Marshall *et al.*, 2020), and changes in voltinism (Van Dyck *et al.*, 2015; Forrest *et al.*, 2019; Kerr *et al.*, 2020) in various insect species.

Zygogramma bicolorata Pallister (Coleoptera, Chrysomelidae) is an effective biological control agent for Parthenium hysterophorus L. (Asteraceae), an invasive herbaceous weed with a pan-tropical distribution. The weed affects grass productivity and endemic biological diversity and causes different medical symptoms in humans (Patel, 2011; Jayaramiah et al., 2017). From a biological control perspective, the thermal performance of mass-reared insects facing novel environments upon release in the wild has long been a source of unease (Enserink, 2007; Terblanche and Chown, 2007; Chidawanyika and Terblanche, 2011; Sørensen et al., 2013; Terblanche, 2014). Several studies have argued that mass-reared insects, typically kept under constant optimal environments, may struggle under field conditions (Enserink, 2007; Kristensen et al., 2008; Chidawanyika and Terblanche, 2011). Therefore, it is imperative to understand the physiological responses to thermal variation in insects used in biological control. It may help optimize rearing and release protocols to enhance field performance (Terblanche, 2014).

Although, studies on consumption and utilization of food by Z. bicolorata have been studied by Bhumannavar and Balasubramanian (1998) and Omkar and Afaq (2011) but very few studies have shown the effect of temperature on the feeding efficiency (Afaq, 2012), development and survival (Omkar et al., 2008), mate guarding behaviour (Bhaisare and Chaudhary, 2023), plant mediated effects of temperature and CO<sub>2</sub> on biocontrol (Kumar et al., 2021), effect of temperature and altitude on feeding attributes (Bhusal et al., 2020), and heat tolerance (Chidawanyika et al., 2017) of Z. bicolorata. Nevertheless, the effect of various rearing thermal conditions on the feeding parameters of Z. bicolorata have not been investigated so far. In the present study, investigated the effects of various thermal conditions on the feeding attributes of *Z. bicolorata*, *i.e.*, consumption index, conversion efficiency, digestibility, and growth rate.

# MATERIALS AND METHODS

Both sexes of Z. bicolorata adults were collected from agricultural fields of Amarkantak ( $22^{\circ}40^{\circ}N$ ,  $81^{\circ}45^{\circ}E$ ), Madhya Pradesh, India. The adults were paired randomly in plastic Petri dishes ( $9.0 \times 1.5$ cm) and allowed to mate until natural disengagement and reared under controlled abiotic conditions (i.e. temperature:  $25\pm2^{\circ}C$ ; humidity:  $65\pm5\%$ ; photoperiod: 14L:10D) in BOD incubators (REMI CHM-16 Plus). Beetles were provided with fresh leaves of *P. hysterophorus* daily. Eggs laid were collected daily and used for further experimentation.

Batches of (100 eggs per temperature) eggs were collected from the stock and reared till adult maturity at constant temperatures of 15, 20, 25, 30 and 35°C separately at each temperature regime in the BOD incubator with the same abiotic conditions (humidity 65±5%; photoperiod 14L:10D). Afterward, preweighed (Digital weighing balance Model: Aczet-CY223C) adult females were kept with preweighed leaves for 24 hours under respective thermal conditions. After 24 hours, the adult was transferred to a fresh Petri dish, concurrently, the adult biomass, the weight of faeces, and remaining unfed Parthenium leaves were recorded. Each experiment was replicated ten times and the consumption rate, conversion of ingested food, digestibility, conversion of digested food, and growth rate of the adults at different reared thermal conditions were calculated (Waldbauer, 1968).

Consumption index is the consumption made based on the intake rate relative to the animal's mean weight during the feeding period and was calculated as:=

Fresh or dry weight of food eaten	
$(Duration \ of \ feeding \ period) X (Mean \ fresh \ or \ dry \ weight \ of \ animal \ during \ feeding \ respectively \ animal \ during \ feeding \ respectively \ for \ respectively \ for \ respectively \ feeding \ respectively \ respectively \ during \ feeding \ respectively \ respectivel$	ng)
Conversion of ingested food is the efficiency of conversion of ingested food to body substance are was calculated as = $_{\text{Weight gained}}$ v100	of nd
Weight of food eaten	

Digestibility was calculated as =

Weight of food ingested-Weight of feces X100

Weight of food eaten

Conversion of digested food is the efficiency with which digested food is converted to body substance and was calculated as: =

Weight gained Weight of food ingested-Weight of feces

Growth rate =

Fresh mass gain by adults (mg) (Feeding duration)X(Mean biomass of adults)

The data collected on consumption index, conversion of ingested food, absolute digestibility, conversion of digested food, and growth rate were checked for normality with the help of Kolmogorov-Smirnov's test, which revealed normal distribution. Data were subjected to one-way ANOVA followed by Tukey's post hoc honest test of significance. All statistical analyses were done using MINITAB-16 statistical software (Minitab Inc., State College, Pennsylvania, USA).

### **RESULTS AND DISCUSSION**

At the extreme thermal conditions (15 and 35°C), the death of different immature stages (larvae, pupae and under-develop adults) was observed (Table 1). On the other hand, thermal conditions (20, 25 and 30°C) significantly influenced the consumption index (P=0.040, F=68.47, df=2), conversion of ingested food (P=0.004, F=6.16, df=2), absolute digestability (P=0.002, F=10.38, df=2), conversion of digested food (P=0.025, F=3.96, df=2), and growth rate (P=0.012, F=5.28, df=2). The maximum consumption index (41.28±3.54 mg) was observed at 25°C whereas it was minimum (0.02±0.00 mg) at 30°C (Fig. 1). The conversion of ingested food was maximum (17.52±6.23 mg) at 20°C whereas it was minimum (2.46±1.45 mg) at 25°C (Fig. 2). The conversion of digested food was maximum  $(41.16\pm23.29 \text{ mg})$ at 20°C and it was minimum (2.93±1.68 mg) at 25°C (Fig. 4). However, absolute digestibility and growth rate increased with increasing temperature from 20 to 30°C (Figs. 3, 5). In the present study, extreme thermal conditions (15 and 35°C) were not tolerated by the larval and pupal stages and newly emerged adults, leading to death at the immature stages. The intolerance of thermal shock may be because there was a decrease in the number of obligate bacterial endosymbionts, which are responsible for the thermal tolerance of insect host species (Zhang et al., 2019). Experimental beetles were collected from a geographical area where temperature ranged from 15°C to 30°C with an average of  $20 \pm 2^{\circ}$ C throughout the year (Malviya and Dwivedi, 2015). So, the beetle might have adopted this temperature range through epigenetic changes. Temperature is one of the factors for changes in the genome at the epigenetic level (Richard et al., 2019). The physiology and biochemical activities of the beetles are adversely affected by either a range of positive or negative temperature variations.

Consumption index increased from 20°C to 25°C and then decreased at 30°C. This might be because the energy available for activities other than cellular maintenance, such as movement, feeding, or digestion, drops rapidly at high temperatures. This often lead to lower consumption rates at high temperatures (Somero, 2011). Levesque *et al.* (2002) reported a similar pattern of the consumption index in *Malacosoma disstria*. Apart from this, Lemoine *et al.* (2014) investigated the relationship between food consumption and temperature in phytophagous insects which revealed that food



Fig. 1 Effects of temperature on consumption index (Values are Mean± SE; Small letters represent the comparisons of mean between the treatments; Similar letters indicate lack of significant difference)



Fig. 2 Effect of temperature on conversion of ingested food (Values are Mean± SE; Small letters represent the comparisons of mean between the treatments; Similar letters indicate lack of significant difference)



Fig. 3 Effect of temperature on absolute digestibility (Values are Mean± SE; Small letters represent the comparisons of mean between the treatments; Similar letters indicate lack of significant difference)

intake increased as temperature increased up to a certain range. Many studies reported that consumption rates of insects increase to a certain extent with increasing temperature, and after that, the consumption rates vary according to the fluctuation of the temperature (Niu *et al.*, 2003; Yee and Murray, 2004; Rall *et al.*, 2010).

Maximum conversion efficiency was recorded at 20°C than other thermal conditions. Several studies also suggested that with increasing temperature, the food conversion efficiency of adults initially increased to an optimal level and then decreased with a further increase in temperature (Bhusal *et al.*, 2020). The findings of the present study suggest that 20°C might be the optimal temperature for this beetle. However, temperatures above the optimal levels might induce thermal stress reducing its

conversion efficiencies. Similar trends also have been reported in coccinellid beetles (Omkar and Kumar, 2016).

Absolute digestibility increased positively from 20°C to 30°C. This increase in digestibility with temperature might be because of the increase in the metabolic rate of Z. bicolorata. Similar results have been documented by Levesque et al. (2002) and Hegazi and Schopf (2009) in Malacosoma disstria and Spodoptera littoralis (Boisd.). In contrast, the conversion of digested food was maximum at 20°C and minimum at 25°C. At 20°C, the beetle might have efficiently converted the food into nutrients needed for physiological functioning. The above finding suggest that this temperature might be optimal for feeding and converting food material. Similar results have also been recorded in the forest tent caterpillar moth and African cotton leafworm (Hegazi and Schopf, 2009; Levesque et al., 2002).

Growth rate was negligible at 20°C, but increased in temperature 25°C to 30°C, suggesting that the metabolic rates of *Z. bicolorata* adults increased with the temperature which might have stimulated the growth of the beetles. A similar trend has also been reported in the beetle, *Alphitobius diaperinus*, which grew slower at low temperature and faster at high temperature (Bjorge *et al.*, 2018).

In conclusion, the results of the present study suggest that temperature significantly modulated this



Fig. 5 Effects of different thermal conditions on relative growth rate (Values are Mean $\pm$  SE; Small letters represent the comparisons of mean between the treatments; Similar letters indicate lack of significant difference



Fig. 4 Effects of different thermal conditions on Conversion of digested food (E.C.D.) (Values are Mean± SE; Small letters represent the comparisons of mean between the treatments; Similar letters indicate lack of significant difference

beetle's feeding attributes. Using the nutritional indices consumption index, absolute digestibility, and efficiency of conversion of digested food, the capacity of *Z. bicolorata* to consume and utilize food can be described in three steps: (i) feeding activity, (ii) digestion and (iii) efficacy to assimilate the digested food. This sequence demonstrates the conversion of foodstuff into the body substance of the phytophagous beetles.

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Stages	Temperature 15 °C		Temperature 15 °C Temperature 35 °C		
Larva	Dorsal view	Lateral view	Dorsal view	Lateral view	
Pupa	Mostly pupation did not occur. If occurred, under-developed adults formed		No pupation observed		
Adults		Under-developed adult	Adults did not emerge		

Table 1. Effects of extreme thermal conditions on different developmental stages of Zygogramma bicolorata

Parthenium Beetle *Zygogramma bicolorata* Pallister. Indian Journal of Entomology 1–6.

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