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Feeding potential of *Zygogramma bicolorata* (Pallister) on *Parthenium hysterophorus* (Linneaus) at different temperatures in Odisha

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Abstract
Laboratory studies on the "Feeding potential of *Zygogramma bicolorata* on *Parthenium hysterophorus* at different temperatures*"* was taken up in Department of Entomology, Institute of Agricultural Sciences, SOADU during 2019-20. It was observed that the feeding potential of different stages of the insect was highest at 30 ^{0}C followed by 25 ^{0}C. But at 20 ^{0}C and 35 ^{0}C, the parameters showed declining trends indicating that 25-30 ^{0}C is the best for rearing of these predatory beetles in the laboratory. As evidenced from the experiments, it was seen all stages like the second, third and fourth instars as well as the male and females also less at 20 and 35 ^{0}C whereas, it was higher at 25 and 30 ^{0}C. The first instar larvae consumed 2.5mg parthenium leaves at 20 ^{0}C whereas, the corresponding figures were 2.8 and 3.1 mg at 25 and 30 ^{0}C. Similarly, the feeding was higher at 25 and 30 ^{0}C for second, third and fourth instar larvae and adults. Therefore, it was evidenced that 25-30 ^{0}C is the best temperature for rearing of these biocontrol agents and at this temperature also the feeding potential was the highest.

Keywords: *Parthenium hysterophorus, Zygogramma bicolorata* and feeding potential

Introduction
Parthenium hysterophorus L. is a highly invasive plant of global significance. It is a herb of geotropically origin which now has spread to many parts of the world [1]. The weed was accidentally introduced to India in 1955 through the imported food grains and at present it has invaded throughout India in about 35 million hactares of land [2]. It is notorious for causing allergic reactions [3] besides a threat to biodiversity and loss of crop productivity [4] management of Parthenium. The Mexican beetle, Zygogramma bicolorata Pallister (Coleoptera: Chrysomelidae) an effective bio-control agent of P. hysterophorus, was introduced in 1983 from Mexico. In India the biocontrol program of parthenium was initiated in 1984 in Bangalore by the introduction of leaf-feeding beetle, *Zygogramma bicolorata* Pallister (Chrysomelidae: Coleoptera) from Mexico [5]. Both adults and larvae of Z. bicolorata can feed on leaves, terminal buds and leaf blades of parthenium. *Z. bicolorata* causes a 98% reduction in flower production during the wet season [6] and one adult per plant is capable of causing skelentonization of leaves within four to eight weeks [7] in India. Although manual and chemical methods are effective strategies to control the weed in agricultural fields, but these are not economical in pastures and large natural areas or wastelands [8]. Biological control of Parthenium weed is considered to be the most cost effective, environmentally safe and ecologically viable method [9]. Keeping in view the problems of Parthenium weed in Odisha, the investigation on feeding potential of *Zygogramma bicolorata* was undertaken.

Materials and Methods
Studies were conducted at Laboratory, Department of Agricultural Entomology, Institute of Agricultural Sciences, SOA Deemed to be University, Bhubaneswar with ten replications.

Maintenance of stock culture of *Z. bicolorata*
The adults of *Z. bicolorata* were collected from the fields. The Beetles were confined in transparent glass jar (10 cm in height and 5 cm in diameter) and provided with parthenium branches with fresh leaves as food. The leaves were provided in the form of bouquets, with their cut ends covered with moist cotton swab to prevent the leaves from quick drying. The jars were covered with white muslin cloth and secured with rubber band.

Food was changed daily. *Z. bicolorata* laid eggs on the under surface of leaves and under surface of cotton cloth. They were removed with the help of fine camel hair brush and scissor and placed in separate petridishes (110mm) for further rearing. Care was taken to avoid mechanical injury during transfer of eggs. On hatching, larvae were also provided with sufficient leaves of parthenium as a food. Continuous supply of parthenium leaves was made for maintaining laboratory culture of *Z. bicolorata*. Full grown larvae were provided with moistened soil for pupation.

The beetles were reared for two generations in laboratory. Tender leaves of parthenium was changed daily and the third generation was used for studying the biology of the *Zygogramma* beetle in a BOD incubator. The mating pair was kept in the Petri plates of dimension (1.5 x 8 cm). The adults were fed with mixture of tender leaves, old leaves, and inflorescence. Rest of the beetles were kept in a transparent plastic jar (10 cm in height and 5 cm in diameter). One hundred laid eggs were removed with the help of fine camel hair brush and placed separately in petridishes (1.5 cm depth x 8 cm height). Proper attention was given for any mechanical injury during transfer of eggs. On hatching, early instar larvae were fed with tender leaves of parthenium as a food. Continuous supply of parthenium leaves was given to the larvae for preserving the culture *Z. bicolorata*. The larvae molted three times and the instars were judged with the help of exuvia of the larva that was observed daily in the morning hours. Along with recording the incubation period and period between the instars, the measurements of the eggs and the larvae were studied under ocular and stage micrometres. Upon attening pre-pupal stage, the beetles were transferred to glass jars containing a 5cm layer of loose sandy soil for allowing pupation. They entered inside soil and pupated.

Effect of temperature on *Z. bicolorata*

The biological studies were undertaken at four temperature regimes i.e. 20,25,30 and 35 ^{0}C in a BOD incubator in the laboratory and the effect of temperature was evaluated in respect of morphometry, duration of life stages, weight of the different life stages and food consumption of the larvae and adults. Ten replications were maintained with ten individuals taken in each petridish.

Feeding potential of *Z. bicolorata* on *P. hysterophorus*:

The study of feeding potential were carried out in the laboratory during September, November 2019 at 28.42 ± 1.43 ^{0}C and 60.74 ± 2.36 per cent relative humidity. Set of 50 first instar larvae and newly emerged adults were used for study.

Larva

A set of ten first instar larvae were confined in the petridishes (110mm). The larvae were provided with leaves of parthenium on weight basis during morning hours (8:00 to 10:00 am). For calculation of feeding potential of grub, fresh food was supplied to each container with 10 numbers of larvae on weight basis.

Adult

The newly emerged beetles of *Z. bicolorata* were kept in glass jar (10 cm in height and 5 cm in diameter).

Statistical Analysis

A set of ten adults were provided with leaves of parthenium on weight basis during morning hours. The calculation of feeding potential of adult, fresh food was supplied to each container with 10 numbers of adults on weight basis. The weight of consumed food was calculated by subtracting the weight of remaining food after feeding in 24 hours and control from the weight of supplied food. Observations data taken were calculated in descriptive statistical analysis method with estimation of mean and standard deviation variance.

Results and Discussion

The feeding potential of different stages of *Z. bicolorata* was evaluated at 20, 25, 30 and 35 ^{0}C and results are as follows. Perusal of data in Table-1 indicated that, at 20 ^{0}C, the first instar larvae consumed the parthenium leaves in the range of 2-3 (2.5±0.1) mg. The second instar consumed at a higher rate and recorded 6-8 (6.9-0.2) mg. The third instar larvae were more voracious and consume leaves at faster rate and the consumed leaves by them was to the tune of 12-14 (12.8±0.6) mg. In the fourth instar, however, it was noticed that the consumption rate declined and it was observed to be 12-13 (12.0±0.01) mg. This may be due to the approaching stoppage in the feeding as the larvae entered the pre-pupal stage. Adult males consumed less in comparison to the females which may be attributed to the larger size and life spam of the females. The males fed to the tune of 12-13 (12.1±0.2) mg, whereas, the females were found to be consuming more in comparison to the males and recorded 14-15 (14.1±0.03) mg. Our study was in agreement with Chandravanshi (2018) [10] studied that the food consumption of Mexican beetle, *Zygogramma bicolorata* was conducted under laboratory conditions at 25-30°C and 60 ± 5% RH. The parthenium leaves consumed by first instar larvae at 25 ^{0}C was in the range of 2-3 (2.8±0.2) mg. and the second instar consumed at a higher rate and recorded 7-8 (7.2±0.4) mg. The third instar larvae consumed more voraciously and at more faster rate and the recorded consumed leaves by them was to the tune of 13-14 (13.1±0.8) mg. In the fourth instar, 12-13 (12.3±0.04) mg. This may be due to the approaching stoppage in the feeding as the larvae entered the pre-pupal stage. Adult males consume less in comparison to the females which may be attributed to the larger size and life spam of the females. The males were observed to be feeding to the tune of 12-14 (12.15±0.5) mg, whereas, the females were found to be consuming more in comparison to the males and recorded 14-16 (14.6±0.07) mg. Our finding are in corroborate with Swamiappan (1997) [11] for the feeding behavior of *Z. bicolorata.* (Table-1).

At 30 ^{0}C, the first instar larvae consumed the parthenium leaves in the rage of 3-4 (3.1±0.5) mg. the second instar consumed at a higher rate and recorded 7-9 (7.6-0.7) mg. Feeding the third instar larvae which were found to be more voracious and consumed leaves at more faster rate and the consume leaves by them was to the tune of 13-14 (13.6±0.12) mg. In the fourth instar, it was noticed that the consumption rate declined to 13-14 (13.1±0.07) mg. This may be due to the approaching pre-pupal stage where larvae will be entering a brief diapause. Adult males consumed less in comparison to the females which may be attributed to the larger size and life spam of the females. The males fed to the tune of 12-14 (12.9±0.9) mg, whereas, the females were found to be consuming more in comparison to the males and recorded 15-16 (15.1±0.09) mg. (Table-2) Our findings are in corroboration with Arthanari *et al.,* (2018) [12] that the feeding behavior of *Zygogramma bicolorata* beetles on *Parthenium hysterophorus.* Consumption of food by first instar larvae at

35 ^{0}C in the rage of 2-4 (2.9±0.3) mg. The second instar consumed at a higher weight and recorded 7-9 (7.3-0.5) mg. The third instar larvae were more voracious and consume leaves at more faster rate and the consume leaves by them was to the tune of 13-14 (13.3±0.10) mg. The fourth instar, however, it was noticed that the consumption rate decline and observed to be 12-13 (12.9±0.06) mg. This may be due to the approaching stoppage in the feeding as the larvae entered the pre-pupal stage. Adult males consume less in comparison to the females which may be attributed to the larger size and life spam of the females. The males fed to the tune of 12-14 (12.6±0.7) mg, whereas, the females were found to be consuming more in comparison to the males and recorded 14-16 (14.9±0.08) mg. (Table-2)

Effect of temperature on *Z. bicolorata*

The weight of various life stages of Z. *bicolorata* at 20 ^{0}C is presented in Table-3. Eggs weighed 0.1-0.2(0.1±0.02) mg. The first instar larvae exhibited weight of 0.2-0.3 (0.25±0.2) mg at 20 ^{0}C. Second instar larvae were found to be varying between 4.0-6.0 (4.83±1.94) mg in weight whereas the third instar larvae weighted 14.0-16.0 (14.32±1.69) mg last instar, i.e., fourth instar larvae were 31.0-32.0 (30.80±1.82) mg. The pupae weighted slightly weighted than the larvae registering 37.0-43.0 (37.8±3.66) mg weight. Adult males were lighter in weight showing 30.0-33.0 (30.4±2.89) mg weight whereas the females were heavier with 41.0-47.0 (41.7±5.66) mg. Our study was in agreement with Geetha *et al.,* (1993) [13] about the study of the weight of *Z. bicolorata* at different temperatures. Depicted data in Table-3 to revealed that, at 25 ^{0}C, the eggs weight 0.1-0.2 (0.17±0.02) mg which were slightly heavier than at 20 ^{0}C. Similarly, first instar larvae were 0.3-0.5 (0.28±0.04) mg in weight and the second instar were substantially heavier with 4.0-7.0 (4.87±1.96) mg in weight. Subsequently the third instar increased significantly and weighed 14.0-16.0 (14.7±1.73) mg and the fourth instar also increased rapidly in weight regime of 31.0-33.0(31.6±1.88) mg. The pupae were heavier than the fourth instar with 38.0-44.0 (38.2±4.70) mg weight. Adult males weighed less than the pupae showing the weight of 31.0-35.0 (31.6±2.98) mg in weight whereas the female were heavier with 42.0-48.0 (42.4±5.74) mg in weight Omkar *et al.,* (2008) [14] studied the effect of different constant temperatures on *Z. bicolorata*, which is similar to our study.

It was observed that, at 30 ^{0}C the weights of eggs were 0.2-0.5 (0.20±0.04) mg. The first instars larvae were 0.3-0.8 (0.31±0.07) mg in weight. The second instars and third instars weight were 5.0-8.0 (5.01±2.03) and 15.0-17.0 (15.1±1.80) mg respectively. The fourth instars were slightly less weight than third instars having weight 32-34 (32.2±1.92) mg. The pupae were 40-60 (40.1±5.79) in weight. Adult males were lighter in weight showing 32.0-36.0 (32.1±3.04) mg weight whereas the females were heavier with 42.0-48.0 (42.9±5.79) mg. (Table-3) Our findings are in agreement with Siddhapara *et al*., (2012) [15]. The perusal of data is Table-4 indicated that, at 35 ^{0}C the weights of eggs were 0.1-0.4 (0.19±0.03) mg. The first instars larvae were 0.3-0.7 (0.30±0.06) mg in weight. The second instars and third instars weight were 4.0-7.0 (4.94±2.00) and 14.0-16.0 (14.9±1.99) mg respectively. The fourth instars were slightly less weight than third instars having weight 31-33 (31.9±1.91) mg. The pupae were 39-45 (39.4±5.77) mg in weight. Adult males were lighter in weight showing 31.0-35.0 (31.9±3.00) mg weight whereas the females were heavier with 42.0-48.0 (42.6±5.77) mg. Our study was in agreement with Omkar *et al.,* (2009) [16]. It was observed that the weight of different life stages was dependent upon the feeding potential. As in case of feeding, the maximum weight was recorded at 30 ^{0}C and minimum at 20 ^{0}C. Our finding is in agreement with the findings of Jaiswal and Ganguli (2020) [17] on the weight of the beetle.

Table 1: Consumption of leaves (mg) by *Z. bicolorata* at 20 ^{0}C and 25 ^{0}C

	20 ^{0}C		25 ^{0}C	
Stage	**Range**	**Consumed**	**Range**	**Consumed**
1st instar	2-3	2.5 ± 0.1	2-3	2.8 ± 0.2
2nd instar	6-8	6.9 ± 0.2	7-8	7.2 ± 0.4
3rd instar	12-14	12.8 ± 0.6	13-14	13.1 ± 0.8
4th instar	12-13	12.0 ± 0.1	12-13	12.3 ± 0.4
Adult Male	12-13	12.1 ± 0.2	12-14	12.5 ± 0.5
Adult Female	14-15	14.1 ± 0.3	14-16	14.6 ± 0.7

Table 2: Consumption of leaves (mg) by *Z. bicolorata* at 30 ^{0}C and 35 ^{0}C

	30 ^{0}C		35 ^{0}C	
Stage	**Range**	**Consumed**	**Range**	**Consumed**
1st instar	3-4	3.1 ± 0.5	2-4	2.9 ± 0.3
2nd instar	7-9	7.6± 0.7	7-9	7.3 ± 0.5
3rd instar	13-14	13.6 ± 0.12	13-14	13.3 ± 0.10
4th instar	13-14	13.1 ± 0.7	12-14	12.9 ± 0.6
Adult Male	12-14	12.9 ± 0.9	12-14	12.6 ± 0.7
Adult Female	15-16	15.1 ± 0.9	14-16	14.9 ± 0.8

Table 3: Weight (mg) of different life stages of *Z. bicolorata* at 20 ^{0}C and 25 ^{0}C

	20 ^{0}C		25 ^{0}C	
Stage	**Range**	**Mean ± SD**	**Range**	**Mean ± SD**
Egg	0.1-0.2	0.14±0.02	0.1-0.2	0.17±0.02
1st instar	0.2-0.3	0.25±0.02	0.2-0.5	0.28±0.04
2nd instar	4.0-6.0	4.83±1.94	4.0-7.0	4.87±1.96
3rd instar	14.0-16	14.3±1.69	14.0-16.0	14.7±1.73
4th instar	30.0-32.0	30.8±1.82	31.0-33.0	31.6±1.88
Pupa	37.0-43.0	37.8±5.66	38.0-44.0	38.2±5.70
Adult Male	30.0-33.0	30.4±2.89	31.0-35.0	31.6±2.98
Adult Female	41.0-47.0	41.7±5.66	42.0-48.0	42.4±5.74

Table 4: Weight (mg) of different life stages of *Z. bicolorata* at 30 ^{0}C and 35 ^{0}C

	30 ^{0}C		35 ^{0}C	
Stage	**Range**	**Mean ± SD**	**Range**	**Mean ± SD**
Egg	0.2-0.5	0.20±0.04	0.1-0.4	0.19±0.03
1st instar	0.3-0.8	0.31±0.07	0.3-0.7	0.30±0.06
2nd instar	5.0-8.0	5.01±2.03	4.0-7.0	4.94±2.00
3rd instar	15.0-17.0	15.1±1.80	14.0-16.0	14.9±1.77
4th instar	32.0-34.0	32.2±1.92	31.0-33.0	31.9±1.91
Pupa	40.0-46.0	40.1±5.79	39.0-45.0	39.4±5.77
Adult Male	32.0-36.0	32.1±3.03	31.0-35.0	31.9±3.00
Adult Female	42.0-48.0	42.9±5.79	42.0-48.0	42.6±5.77

Conclusion

The average rate of feeding *Z. bicolorata* on parthenium was also substantially influenced by the fluctuation of temperature. The first instar larvae fed 2.5 mg, 2.8 mg and 3.1 mg at 20 ^{0}C, 25 ^{0}C and 30 ^{0}C respectively. Showing increasing trend but at 35 ^{0}C, the food consumption was 2.9. Again, indicating the decreasing trend at higher temperature. Corresponding to the feeding the mean weight of different stages of *Z. bicolorata* also showed significant difference at different temperature. The eggs were 0.14 mg at 20 ^{0}C

whereas, they were 0.17 and 0.20 mg at 25 and 30 ^{0}C respectively. There was a decline at 35 ^{0}C recording 0.19 mg. similarly the mean weight of first instar was 0.25, 0.28, 0.31 and 0.30 mg at 20, 25, 30 and 35 ^{0}C respectively. These observations on different biological parameters, indicated that the beetles thrived and prefer the temperature range of 25 ^{0}C to 30 ^{0}C where they could consume maximum food. The temperature beyond 35 ^{0}C and below 20 ^{0}C were found unsuitable for the beetles. Therefore, the beetles should be reared in the lab between 25 ^{0}C to 30 ^{0}C for mass production to be used in biological control of parthenium.

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