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Morphometric analysis with respect to life stages development of *Zygogramma bicolorata* Pallister (Coleoptera: Chrysomelidae) in lower-temperate region of Tehri Garhwal Himalaya Uttarakhand, India

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Abstract
The present study was based on morphometric study with respect to feeding with respect to their development of life stages of *Z. bicolorata*. *Z. bicolorata* has four larval instar which feed on the parthenium leaves for food. Mean length and width of egg was 1.17±0.05 mm and 0.53±0.05 mm, mean length, width and head capsule of 1st instar larva was 1.72±0.12 mm, 0.73±0.15 mm, 0.55±0.04 mm respectively. While the mean length, width and head capsule of 2nd instar larva was 2.64±0.17 mm, 1.09±0.16 mm, 0.76±0.04 mm. Whereas, the mean length, width and head capsule 4th instar larva was 6.38±0.20 mm, 2.48±0.18 mm, 1.45±0.08 mm, 4th instar larva undergo pupa stage in the soil and its measurement 6.35±0.38 mm length, 3.23±0.16 mm width recorded. In pupa stage head capsule not clearly differentiate. Adult and male 6.44±0.22 mm length, 3.32±0.24 mm width, and 1.62±0.07 mm width. while female was large than male as 7.85±0.25mm length, 3.36±0.32 mm width, 1.80±0.05 mm head capsule width was recorded. The all-body parameters of the beetle increased with respect to increasing food at every stage of life.

Keywords: *Z. bicolorata*, *P. hysterophorus*, morphometry, Tehri Garhwal Himalaya

Introduction
Parthenium hysterophorus L. is an invading, noxious, notorious, fast growing herbaceous weed plant. It is a major crop of waste land, agricultural land, forest land, road sides etc. due to presence of some allelopathic chemicals weed is harmful for agricultural crops, natural plant diversity. It is hazardous for animals and human health due to presence of some allergic chemicals. Nowadays, it is spreading all over the world basically tropical, sub- tropical to temperate and lower temperate regions including India. So, it is a weed of global significance (Adkin and Sowerby 1996, Mahadevappa and Patil (1997); Kumar & Ray 2011, Kumar *et al*., 2022) [1, 19, 17]. Due to its harmful effects management of this weed is very necessary.
Zygogramma bicolorata Pallister is the prominent and significant bio-control agent, feeding on leaves and flowers of *Parthenium* weed. The beetle was first introduced to Australia from Mexico in 1980 (McFadyen and McClay, 1981) [16] and subsequently to India in 1983 (Jayanth, 1987) [13].
This particular beetle was initially introduced by the Indian Institute of Horticultural Research (IIHR), Bangalore, to bio-control parthenium weed (Jayanth and Nagarkatti, 1987) [13]. Within three years of its introduction, the *Zygogramma* beetle reached a significant amount and became abundant (Jayanth and Bali, 1994a; Jayanth and Visalakshy, 1996) [3]. As biological control programs initiatives were started in Jammu and Kashmir in 1989, a large quantity of this beetle was released (Gupta *et al*., 2002) [5]. Since then, beetles have spread throughout an area of more than 9000 km2 and weed suppression is clearly visible in several areas of the state (Gupta *et al*., 2004; Gupta, 2008) [4, 6].
Dhileepan and Senaratne (2009) [3] used a GIS (Geographic Information System)-based map to establish where *Z. bicolorata* is distributed; this map highlights data on the prevalence of *P. hysterophorus* in several Indian states and territories, as well as in Bangladesh, Bhutan, Nepal, and Pakistan. *Z. bicolorata* was found to occur in three separate clusters across various parts of India. Sushilkumar and Ray (2011) [19] estimated the mean body length and weight of male and female *Z. bicolorata* at 5.6-6.5 mm and 30-40 mg, respectively, with longitudinal dark brown lines on the elytra. Present study reveals the morphometric observation development with body parameter of *Z. bicolorata.*

Materials and methods
1. Study area
The experiment was conducted during weed growing season in the laboratory at the room temperature and humidity at Hemvati Nandan Bahuguna Garhwal University (A Central University), SRT Campus Badshahithaul Tehri, Tehri Garhwal Himalaya (Indian Himalayan Region, IHR), Uttarakhand (India) in 2019-2020. District Tehri Garhwal is situated at latitude 30.1˚N to 30.9˚N and longitude 77.9˚E to 79.1˚E. Garhwal region is situated between North latitude 29˚26' 15" and 30˚5' 31" and between East longitude 78˚18' 45" and 80˚8' 0" in North West Himalayas (Atkinson, 1884). Geographical location of experimental site is 30˚23`02.837"N 78˚25`25.633"E and elevation is 1750 m asl surrounded by evergreen mountains and situated near Tehri dam at New Tehri city. The climate is lower temperate, snow falls at least in every winter season. All experiments carried out at room temperature at the laboratory (Fig 7).

2. Collection, Rearing and biology of *Zygogramma*
20 individuals were used to morphometric analysis. *Z. bicolorata* beetles have been collected from field and reared

on the *Parthenium* potted plant, insect cages at the laboratory. 20 matting mating pair released on the potted plant and cages. Female beetles started after matting. All morphometric observation has been done with the help vernier caliper, magnoscope and sporting tools. Correlation coefficient, logistic regression analysis (Pearson-X^2) was applied to formulation of results. In the present study methods followed as reported by Siddhapara *et al.*, 2012; Mehta & Raghuraman (2019) [17] and Jaiswal & Ganguli (2020) [7].

Result and discussion

Morphometric observations of different life stages of *Z. bicolorata* under laboratory conditions showing in Table 1, and Fig 7.

1. First Instar grub

The measurement of the first instar larva was 1.72±0.12 mm length and 0.73±0.15mm width and the head capsule width was0.55±0.04 mm. Linear correlation analysis was performed for finding the relationship between consumed food and length, width, and head capsule width of the first instar grub. The linear fit of the plot with equation is shown in figure. The statistical analysis of the data defines highly significant and strong positive relationship of consumed food by first instar grub with all three parameters. (For length, r=0.95, P<0.05, n=20; for width, r=0.96, p<0.05, n=20; for head capsule width, r=0.96, P<0.05, n=20). The regression statistical analysis of all parameters is shown in table. Our result was similar with Mehta and Raghuraman (2019) [17] with slight variation.

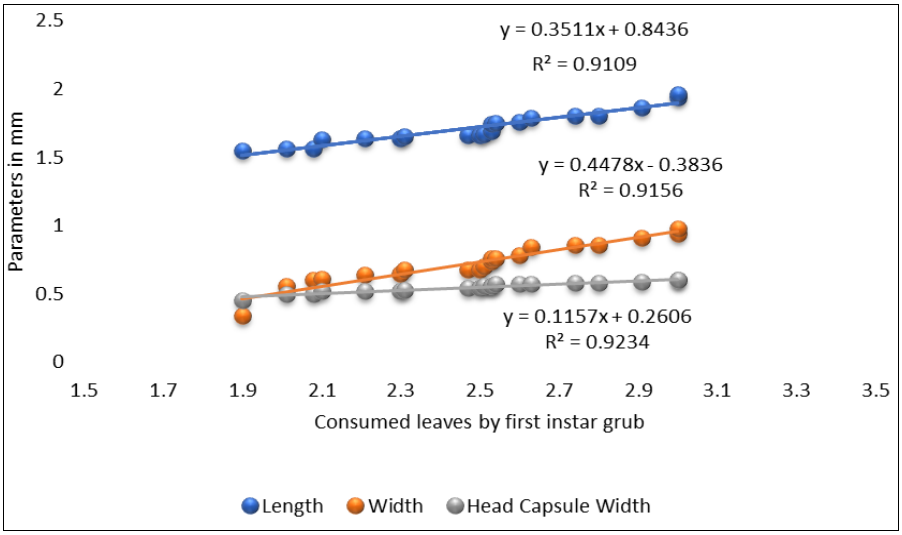

Fig 1: Relationship between body length, body width, and head capsule width of the first instar grubs and consumed food.

2. Second instar grub

The mean length of this grub was 2.64±0.17 mm and mean width was 1.09±0.16 mm and the width of the head capsule was 0.76±0.04 mm.

Linear correlation analysis was also performed for finding the relationship for consumed food and length, width and head capsule width of the second instar grub. The linear fit of the plot with equation is shown in figure. The statistical analysis of the data defines highly significant and strong positive relationship of consumed food by second instar grub with all three parameters. (For length, r=0.96, P<0.05, n=20; for width, r=0.71, p<0.05, n=20; for head capsule width, r=0.96, P<0.05, n=20). The regression statistical analysis of all parameters is shown in fig 2 and all the parameters are highly significant for consumed food.

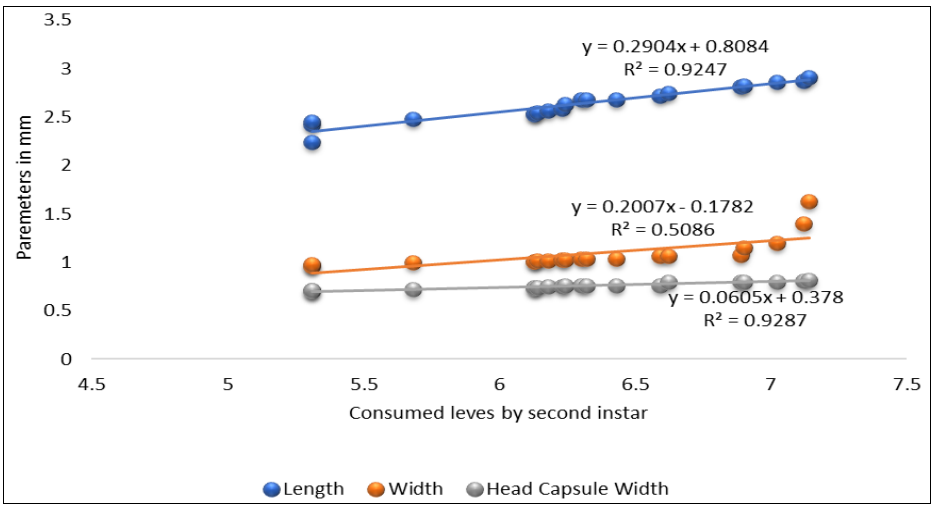

Fig 2: Relationship between body length, body width, and head capsule width of the second instar grubs and consumed foo

3. Third instar grub

The measurement of the third instar larva was 4.56±0.19 mm in length, 2.17±0.26 mm in width, and 1.26±0.17 mm in width of head capsule.

The fourth instar larva appears like that of the third instar larva excluding in size, with a mean6.38 ± 0.20 mm in length 2.48±0.18 mm in width and 1.45±0.08 mm in head capsule with was observed. The circulatory system was visible just below the thin larval skin, which appeared darker, and ran parallel to the faint middorsal yellow longitudinal line. Jayanth & Bali (1993) [11], as well as Parise *et al*., (2010) had previously made similar statements. Mehta and Raghuraman (2019) [17] measured the larva were 4.47 ± 0.39 mm in length and 2.23±0.14 mm in breadth.

Linear correlation analysis was performed for finding the relationship between consumed food and length, width, and head capsule width of the third instar grub. The linear fit of the plot with equation is shown in figure. The statistical analysis of the data defines highly significant and strong positive relationship of consumed food by third instar grub with all three parameters. (For length, r=0.96, P<0.05, n=20; for width, r=0.80, p<0.05, n=20; for head capsule width, r=0.98, P<0.05, n=20). The regression statistical analysis of all parameters is shown in fig 3.

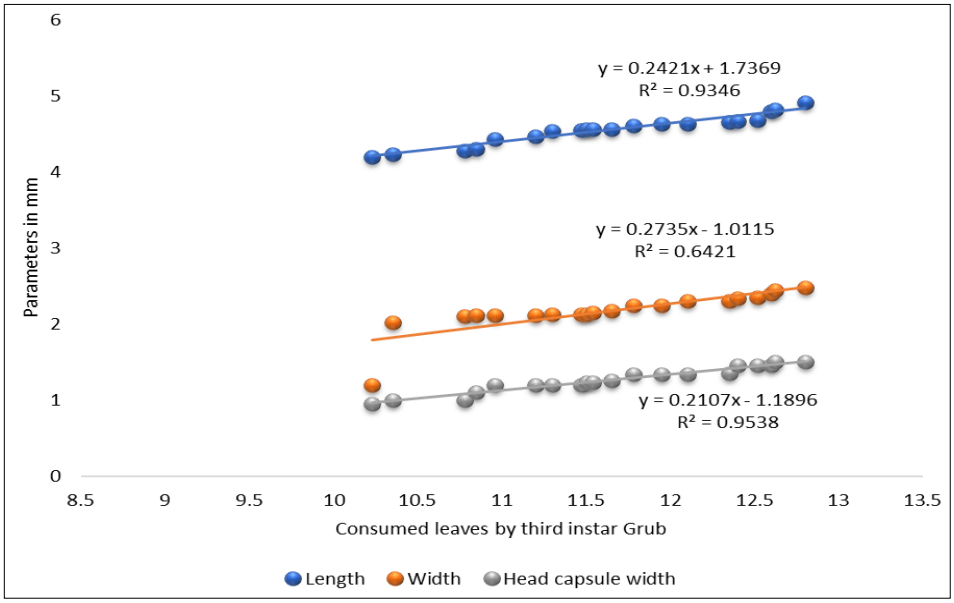

Fig 3: Relationship between body length, body width, and head capsule width of the third instar grubs and consumed food

4. Fourth instar grub

Linear correlation analysis was performed for finding the relationship between consumed food and length, width, and head capsule width of the first instar grub. The linear fit of the plot with equation is shown in figure. The statistical analysis of the data defines highly significant and strong positive relationship of consumed food by fourth instar grub with all three parameters. (For length r=0.89, P<0.05, n=20; for width r=89., p<0.05, n=20; for head capsule width r=0.96, P<0.05, n=20). The regression statistical analysis of all parameters is shown in fig 4.

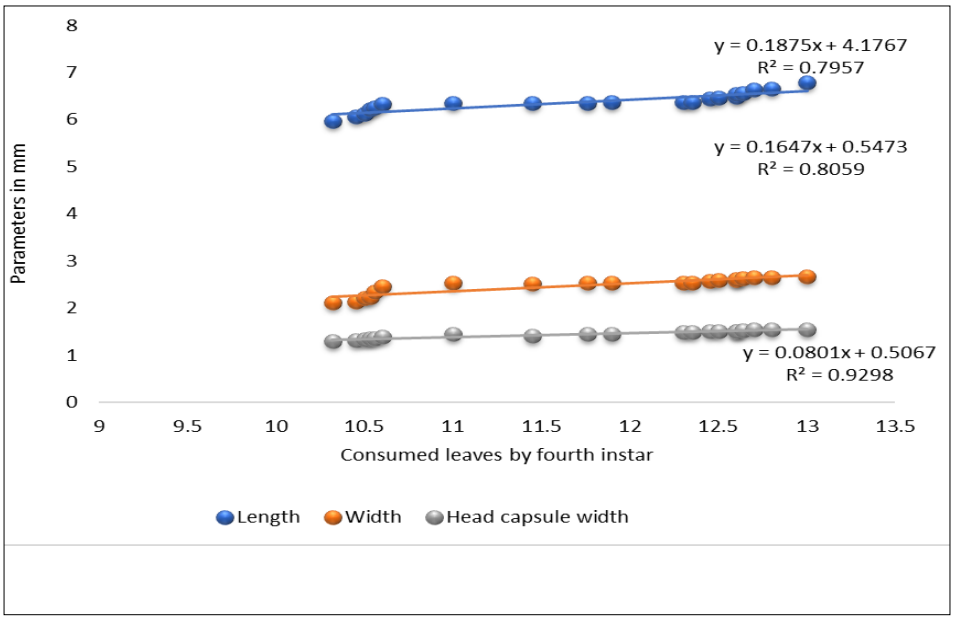

Fig 4: Relationship between body length, body width, and head capsule width of fourth instar grubs and consumed food

5. Adult male

Linear correlation analysis was performed for finding the relationship between consumed food and length, width, and head capsule width of the adult male. The linear fit of the plot with equation is shown in figure. The statistical analysis of the data defines highly significant and strong positive relationship of consumed food by adult male with all three parameters. (For length r=0.91, P<0.05, n=20; for width, r=0.83, p<0.05, n=20; for head capsule width, r=0.98, <0.05, n=20). The regression statistical analysis of all parameters is shown in fig 5.

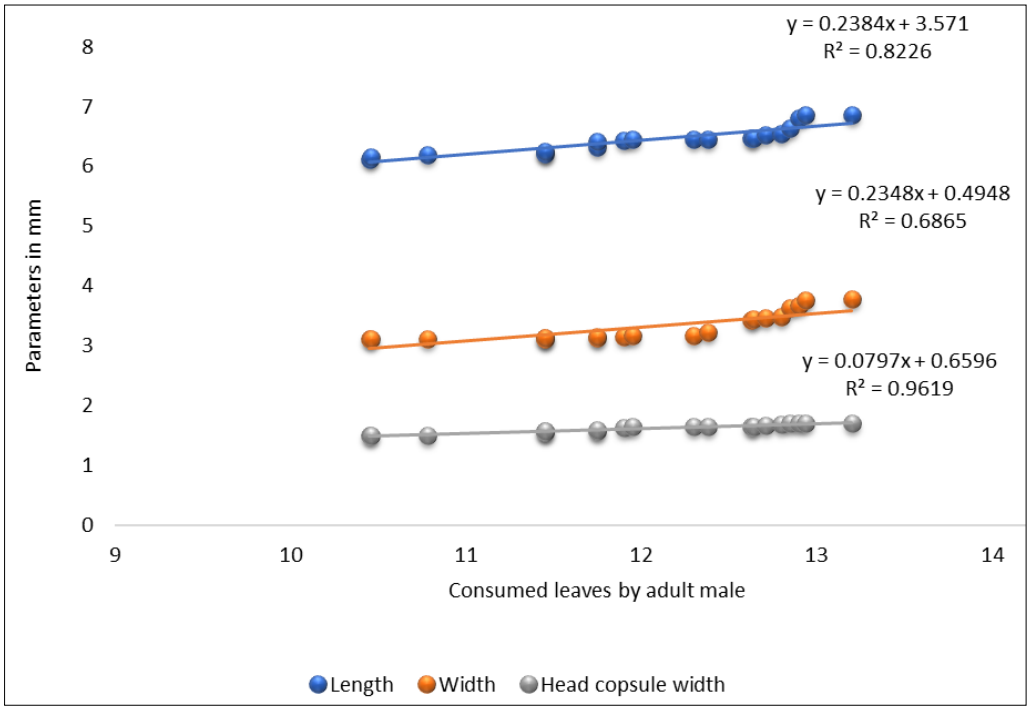

Fig 5: Relationship between body length, body width, and head capsule width of adult male *Z. bicolorata* and consumed food

6. Adult female:

Linear correlation analysis was performed for finding the relationship between consumed food and length, width, and head capsule width of the first instar grub. The linear fit of the plot with equation is shown in figure. The statistical analysis of the data defines highly significant and strong positive relationship of consumed food by first instar grub with all three parameters. (For length r=0.92, P<0.05, n=20; for width r=0.94, p<0.05, n=20; for head capsule width, r=0.83, P<0.05, n=20). The regression statistical analysis of all parameters is shown in fig 6.

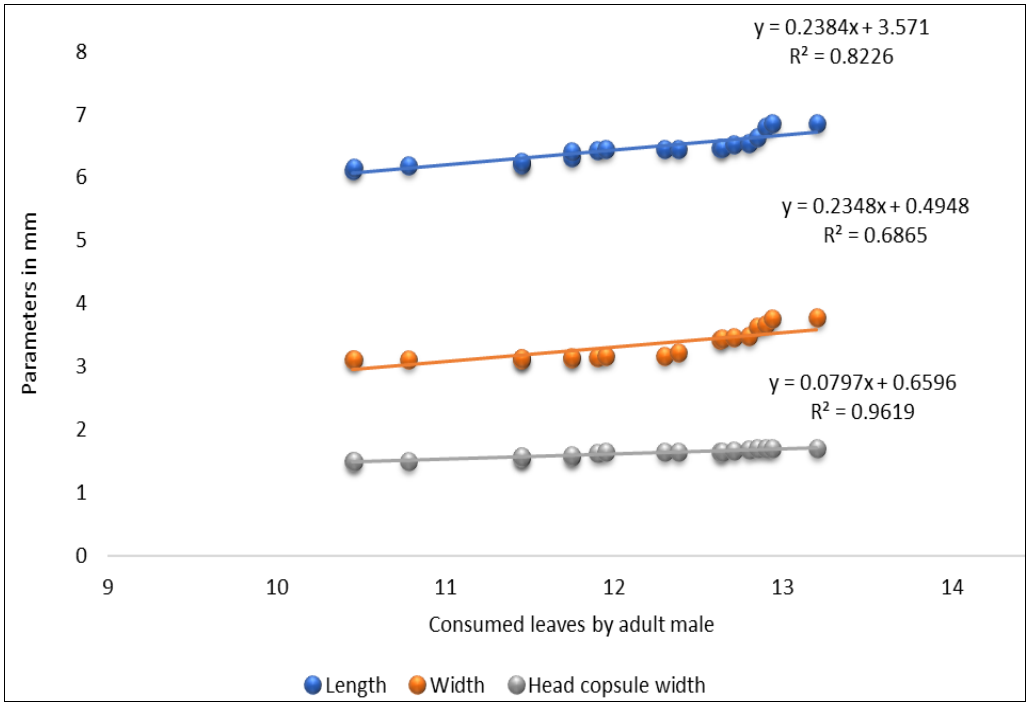

Fig 6: Relationship between body length, body width, and head capsule width of adult female *Z. bicolorata* beetle and consumed food.

Table 1: Morphometric analyses of life stages of *Z. bicolorata* in laboratory

S. N.	Life stages	Length (mm) (Mean± SD)	Width (mm) (Mean± SD)	Head width (mm) (Mean± SD)
1	Egg	1.17±0.05	0.53±0.05	
Larval/ Grub stages				
2	1st instar grub	1.72±0.12	0.73±0.15	0.55±0.04
3	2nd instar grub	2.64±0.17	1.09±0.16	0.76±0.04
4	3rd instar grub	4.56±0.19	2.17±0.26	1.26±017
5	4th instar grub	6.38±0.20	2.48±0.18	1.45±0.08
6	Pupa	6.35±0.38	3.23±0.16	
Adults				
7	Male	6.44±0.22	3.32±0.24	1.62±0.07
8	Female	7.85±0.25	3.36±0.32	1.80±0.05

Source: Primary Data collected in 2019-20

The morphometric observations are slightly different from Siddhapara *et al.*, 2012 in the egg size only. As they reported eggs length 1.51 ± 0.05 mm and width 0.59 ± 0.03 mm, which is slightly larger than with our results. While, all larval stage and adult male and female size is larger than Siddhapara *et al*., 2012. Mehta and Raghuraman *et al.*, also reported similar results for morphometric analysis of *Z. bicolorata*. The similar studies about larvae instar reported by some author discussed further. 1st, 2nd, 3rd, and 4th instars measured 0.55 ± 0.02, 0.71 ± 0.03, 1.07 ± 0.06 and 1.40 ± 0.07 mm in breadth while 1.61 ± 0.14, 2.38 ± 0.26, 4.03 ± 0.40 and 5.63 ± 0.53 mm in length. The four larval instars duration were, 3.02 ± 0.51, 2.88 ± 0.33, 2.40 ± 0.49 and 3.48 ± 0.73 days, respectively reported by Siddhapara *et al*., 2012.While, the mean length (5.72 ± 0.49 mm), and mean breadth (3.44±0.56 mm).

Fig 7: Showing morphometric study with respect to life history of *Z. bicolorata* (Photographs by Nikon 5600D in 2019-20 at laboratory)
Conclusion: *Z. bicolorata* is a classical biocontrol agent used globally for the biological management of *Parthenium* weed.

Conclusion

Zygogramma bicolorata is widely used a classical bioagent for management of *Parthenium hysterophorus* L. weed. *Z. bicolorata* undergoes eggs, larvae (i.e., larval instars i.e., 1st instar, 2nd instar, 3rd and 4th instar grubs), pupa, and adult. The larvae and adult feed on parthenium leave as a food.

Among the all-life stages adult female feed more food and gained more body length followed by adult male, and forth instar larva, third instar larva, second instar larva. Smallest observation recoded with first instar larvae with minimum food eaten by them. The all-body parameters were developed significantly as increasing with food and all observations were positively correlated.

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