



## Detection of two spotted stink bug, *Perillus bioculatus* (Fab.) on *Zygogramma bicolorata* Pallister in the sub-tropical region of Tehri Garhwal Himalaya, Uttarakhand, India

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### Abstract

*Perillus bioculatus* Fab. (Hemiptera: Pentatomidae) bug is the native to North America. It is frequently used as biopesticide to control the pest on potato *Leptinotarsa decemlineata* Say, (Colorado potato beetle). Present study documents on predatory potential of the two spotted stink bug *Perillus bioculatus* for the first time in sub-tropical region of Tehri Garhwal, Uttarakhand. The nymph stages and the adult of this bug were observed to predate on the *Zygogramma bicolorata* (beetle) which is a biocontrol agent for noxious weed *Parthenium hysterophorus* L. Nymphs were found to target the grubs (beetle larva) only while adults predate on both grubs as well adult beetle. The feeding potential of the bug revealed positive correlation with the developmental stages, minimum being exhibited by first instar and maximum (24-26 *Z. bicolorata*/bug) by adults. This predatory behavior of *Perillus bioculatus* on *Zygogramma bicolorata* underpins its harmful impacts and hence can be regarded as limiting factor for the *Parthenium* weed management.

**Keywords:** *Perillus bioculatus* Fab, *Zygogramma bicolorata* P, Feeding potential, Tehri Garhwal Himalaya

### Introduction

The *Perillus bioculatus* Fab (Hemiptera: Pentatomidae); the two-spotted stink bug or double-eyed soldier bug, a native to the North America is known as the most important natural predator of *Leptinotarsa decemlineata* (Say), Colorado potato beetle, (Coleoptera: Chrysomelidae) a destructive pest of potato worldwide in the Nearctic region (Knight 1952, Froeschner, 1988) [7, 5]. *Perillus bioculatus* bug is reported to spread from Mexico to Canada 9 (Goldstein *et al.*, 1996) [6]. After its unsuccessful introduction, it was only cited as an alien species in Europe (Rabitsch, 2008; Protic and Nebojsa, 2012) [10, 9]. However, recent reports recorded this species in many European countries (Elisovetcaia and Derjanschi, 2016) [4] and North India (Prasad and Pal, 2015) [8], where it was observed to feed on the *Zygogramma bicolorata* Pallister (Chrysomelidae- Coleoptera) a Leaf feeding beetle used as bio-insecticide for the management of *Parthenium hysterophorus*, thus is a classical bio- control agent for management of noxious *Parthenium* weed. *Parthenium* on the other hand is exotic, invasive weed; a threat to the agriculture, loss of biodiversity, human and animal health. Keeping the scenario in view, *P. bioculatus* is regarded as beneficial insect for the control of Colorado potato beetle, while harmful for the management of *P. hysterophorus* weed. The present study, records the *P. bioculatus* feeding on *Z. bicolorata* for the first time in the Tehri Garhwal of Indian Himalayan Region (IHR). Since, negligible literature was found on the distribution and feeding biology of *Perillus bioculatus* in the IHR, the present study is first of such kind.

### Materials and Methods

#### Study Area

Systemic field survey for the study of the predatory bug

*Perillus bioculatus* (Fab.) were carried in Tehri Garhwal at Chauras Campus HNBG University Srinagar Garhwal during weed growing season 2019. 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) [1]. Geographical location of experimental site was 31.3° 33.00°N and 78°48' 10.78"E and elevation is 560 m ASL surrounded by evergreen mountains and situated on the bank of Alaknanda river at Srinagar. The climate is sub-tropical. The winter season start from October and continues till the end of February. In summer, maximum temperature reaches up to 40°C May and June while maximum rainfall occurs in the month of August and minimum in January.

### Insect Identification

The insect identification was done at Entomological Section of Forest Research Institute Dehradun, Uttarakhand.

### Experimental Protocol

The experimental protocol for the study of predatory potential of bug *P. bioculatus* was adopted from Prasad and Pal (2015) [8]. *Perillus* bugs were collected from *Parthenium* plant were placed in a setup of mosquito net cages along with *Zygogramma* grubs and adults, *Parthenium* plant served as the substrate for egg laying and food for *Zygogramma*. Grubs and adults were released in cage to serve as food for *P. bioculatus*.

The experiment for the study of predatory potential of nymphs and adults were carried out under natural environmental conditions. For this study mosquito net cage setup were used. After hatching, first instar nymph was found to feed for some hours on succulent stem of *Parthenium* and later start to feed on the grubs the other

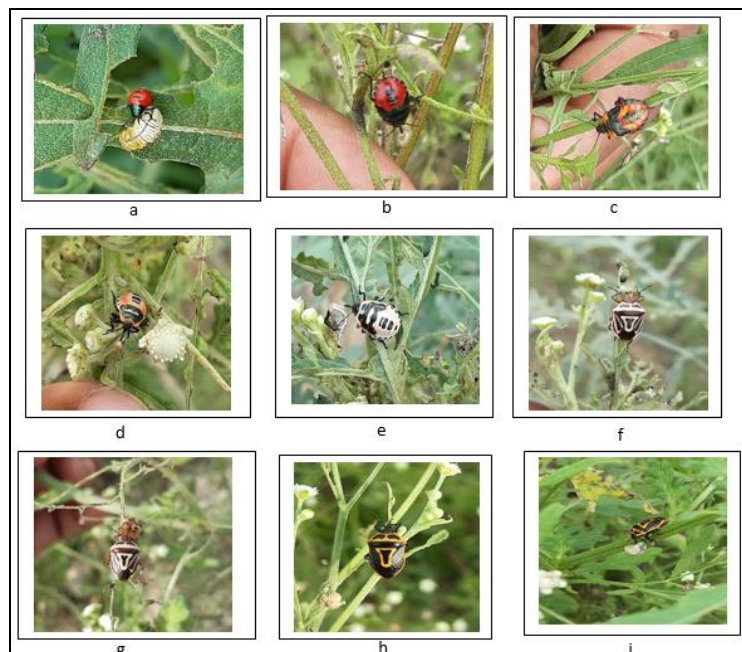
remaining nymphal instars also start to feed on grubs of *Z. bicolorata* and each nymphal stage kept in different mosquito net cage set up. And newly emerged adults were also kept on separate mosquito cage set up and each set up was provided with 10 grubs (mixed size) and adults as food. The daily consumption of *Zygogramma* grub by nymph and adults of *P. bioculatus* were observed and calculated, thereafter the unconsumed grubs left over in the cage were collected daily and calculated for the consumed ones. The actual number of *Zygogramma* grubs was obtained by subtracting the number of *Zygogramma* grubs left over from the total number of *Zygogramma* grubs every day. With similar experimental setup was also prepared for the calculation of adults *Zygogramma* beetles consumed by *P. bioculatus*. Predatory potential (feeding potential) of *P. bioculatus* was subjected to Regression analysis, Correlation was performed between the maturity stages of *P. bioculatus* and the consumed food material.

## Results and Discussion

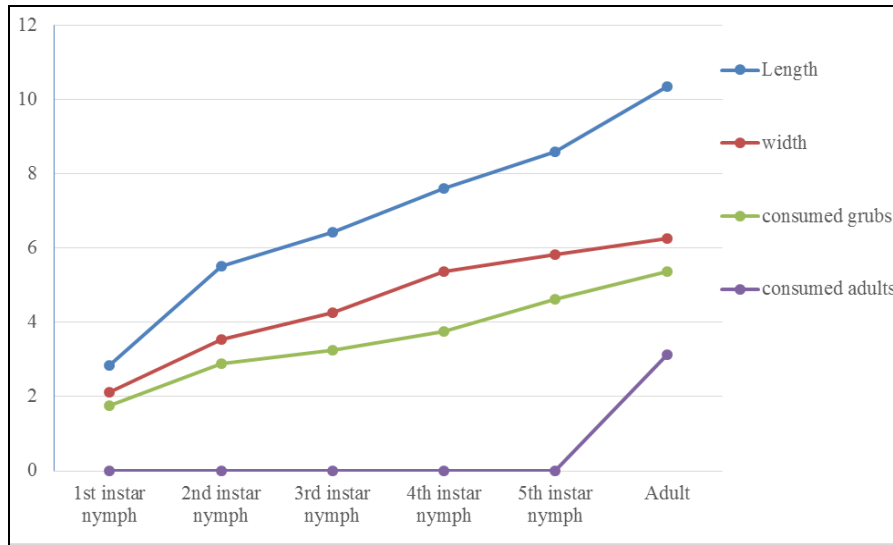
During the extensive survey in *Parthenium* infested field *Z. bicolorata* was found abundantly and significantly defoliating the parthenium as a classical biocontrol agent in the sub-tropical region of Tehri Garhwal Himalaya. *P. bioculatus* was also recorded and found preying on the grubs as well as adult *Z. bicolorata* beetles. Two spotted stink bug *P. bioculatus* adult had a distinct "Y" shaped marking and pronotum and two black spots on the prothorax (Bishop *et al.* 1982). Adult bug start laying eggs in about 5-7 days after matting. The eggs are barrel shaped and laid in batches, containing 25-30 eggs in each cluster. The eggs are laid on the upper surface of the Parthenium leaves. Newly laid eggs were bright green and in a few hours the turn shiny black. The newly hatched nymph emerged from circular hole on the top of the eggs. Eggs were hatched in  $5.13 \pm 0.13$  (Range 5-6 days) days and 1<sup>st</sup> instars (Fig. 1a) begin sucking juice from *Parthenium* stem for few hours and start feeding only on grubs of *Z. bicolorata*. It consumed  $1.75 \pm 0.16$  (range 1-2) grubs in  $3.13 \pm 0.13$  (range 3-4) days. Nymphs were reddish black in color and average size was  $2.85 \pm 0.20$  mm in length and  $2.11 \pm 0.17$  mm in width. They are gregarious in nature. The second instars nymph (Fig. 1b)

stage was completed in  $3.25 \pm 0.16$  (range 3-4) days having average size  $5.52 \pm 0.13$  mm length and  $3.53 \pm 0.17$  mm width, consumed  $2.88 \pm 0.13$  (range 3-4) grubs, while 3<sup>rd</sup> instars nymph (Fig. 1c) took  $3.38 \pm 0.18$  (range 3-4) days to molt in 4<sup>th</sup> instar with  $6.42 \pm 0.10$  mm length  $4.27 \pm 0.10$  mm width in size and consumed  $3.25 \pm 0.18$  (range 3-4) grubs. The 4<sup>th</sup> instar (Fig. 1d) took  $4.13 \pm 0.13$  (range 4-5) days to molt in 5<sup>th</sup> instar nymph and measured  $7.27 \pm 0.10$  mm length and  $5.37 \pm 0.11$  mm width in size and consumed  $3.75 \pm 0.16$  (range 3-4) grubs. The 5<sup>th</sup> and final instar (Fig. 1e) last for  $4.38 \pm 0.18$  (range 4-5) days and its measurement  $8.60 \pm 0.12$  in length and  $5.82 \pm 0.09$  in width and too  $4.38 \pm 0.18$  (4-5) days to change in to adult stage. The nymph changes gradually from reddish- black to pale- yellow or reddish-orange or blackish white with bright reddish- green and adult turned in to distinct color type white and black (Fig 1 f & g) morph and dark yellow and black morph (Fig. h & i). The adult bug measure  $10.35 \pm 0.12$  mm in length and  $6.26 \pm 0.11$  in width. The adult period lasted  $6.26 \pm 0.16$  (Range 6-7) days but in contrary, the adult longevity of *P. bioculatus* bug was reported to be six weeks (Bishop, *et al.* 1982). *Perillus* adult completes life cycle in  $29.63 \pm 0.38$  (28-31) days and consumed  $5.38 \pm 0.18$  (5-6) grubs and  $3.13 \pm 0.23$  (2-4) adults. These results are Similar result was obtained by Prasad and Pal (2015) [8] with some differences, the causes of these differences may be due to different climatic condition, altitudinal variation and different geographical conditions in the study area.

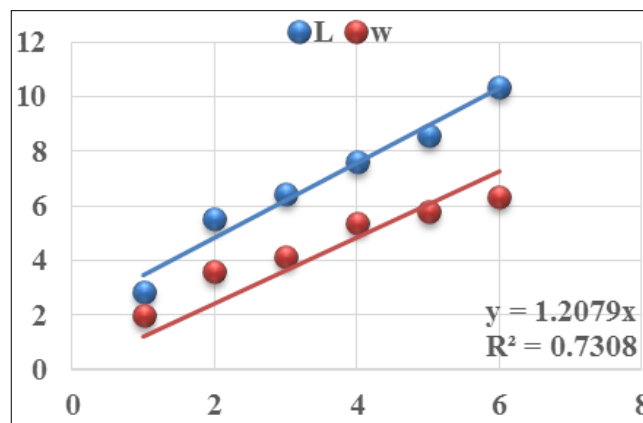
The results of this study showed that the correlation between length, width and consumed food by *P. bioculatus* revealed increase in the consumption of *Zygogramma* with increase in length and width. The length and width increase significantly ( $p > 0.05$ ) from 1<sup>st</sup> instar to adult. The consumption was nearly same upto 3<sup>rd</sup> instar, it increases slightly in the 4<sup>th</sup> and 5<sup>th</sup> instars and sharply increases manifolds in the adult indicating higher feeding potential of the adult insect. Significant differences are also observed in consumed prey among different developmental stages. Regression analysis indicating strong relation ( $R^2 = 0.730$ ) between the length and width in developmental stages of *P. bioculatus*.



**Fig 1:** Showing all nymphal and adult stages of *P. bioculatus* feeding on Grubs and adult *Z. bicolorata*. (a) 1<sup>st</sup> instar nymph, (b) 2<sup>nd</sup> instar nymph, (c) 3<sup>rd</sup> instar nymph, (d) 4<sup>th</sup> instar nymph, (e) 5<sup>th</sup> instar nymph, (f) White and black morph of *P. bioculatus* feeding on Grub *Z. bicolorata*., (g) White and black morph of *P. bioculatus* feeding on Adult *Z. bicolorata*., (h) and (i) Dark yellow and black morph of *Perillus bioculatus* (Fabricius, 1765) [3]



**Fig 2:** Showing increase in consumption of *Zygotogramma* with increase in length and width. The length and width increase significantly ( $p > 0.05$ ) from 1<sup>st</sup> instars to adult. The consumption was nearly same up to 3<sup>rd</sup> instars, it increases slightly in the 4<sup>th</sup> and 5<sup>th</sup> instars and sharply increases manifolds in the adult indicating higher feeding potential of the adult insect. Significant differences are also observed in consumed prey among different developmental stages.



**Fig 3:** Regression analysis indicating strong relation between the length and width in developmental stages of *P. bioculatus*

**Table 1:** Feeding potential of *P. bioculatus* on *Z. bicolorata* (Mean ± SE)

Developmental stages of <i>P. bioculatus</i>	Length and width (mm)		Period/time (days)	Grubs of <i>Zygotogramma</i> consumed/bug	Adults of <i>Zygotogramma</i> consumed/bug
	Length	Width			
Eggs	1.30±0.03 (1.12-1.42)	1.03±0.01 (1.0-1.06)	5.13±0.13 (5-6)	_____	_____
1 <sup>st</sup> instar nymph	2.85±0.20 (2.04-3.54)	2.11±0.17 (1.49-2.63)	3.13±0.13 (3-4)	1.75±0.16 (1-2)	_____
2 <sup>nd</sup> instar nymph	5.52±0.13 (4.92-6.01)	3.53±0.17 (3.01-4.07)	3.25±0.16 (3-4)	2.88±0.13 (2-3)	_____
3 <sup>rd</sup> instar nymph	6.42±0.10 (6.01-4.76)	4.27±0.10 (4.0-4.67)	3.38±0.18 (3-4)	3.25±0.16 (3-4)	_____
4 <sup>th</sup> instar nymph	7.61±0.09 (7.08-7.87)	5.37±0.11 (5.01-5.85)	4.13±0.13 (4-5)	3.75±0.16 (3-4)	_____
5 <sup>th</sup> instar nymph	8.60±0.12 (8.02-8.97)	5.82±0.09 (5.57-6.23)	4.38±0.18 (4-5)	4.63±0.18 (4-5)	_____
Adult	10.35±0.12 (9.98-10.95)	6.26±0.11 (5.94-6.83)	6.25±0.16 (6-7)	5.38±0.18 (5-6)	3.13±0.23 (2-4)
Total period of time taken from egg laying to adult emergence and total consumed grubs and adult			29.63±0.30 (28-31)	21.64±2.98	3.13±0.23
Total consumed <i>Z. bicolorata</i> by <i>P. bioculatus</i>					24.75±0.37 (24-26)

**Conclusion**

*Perillus bioculatus*, an important predator of the Colorado potato beetle, also found to feed on the larvae of *Chrysomela populi*, an important pest in the poplar trees. According to some authors, *P. bioculatus* is a monophag species that feeds exclusively on the Colorado potato beetle. But recent studies showed it also feed on *Z. bicolorata* and *C. populi*. The bug *P. bioculatus* recorded as limiting factor for *Z. bicolorata* and obstructing element for the management of *Parthenium hysterophorus*.

The population of *P. bioculatus* is very lesser in comparison to *Z. bicolorata*. So, impact of *P. bioculatus* on the population *Z. bicolorata* is negligible in the study area. And

in ecosystem, it has been seen some insect are parasitoid on other insects for food, generally bugs feed on beetles and some animals like birds; lizards etc. also feed on these insect and naturally control the population of predatory insects. It is a wonderful natural phenomenon to control the population of animals in an ecosystem. If prey will increase predator will also increase to control the population of each other. So, nature balanced ecosystem itself.

**Acknowledgement**

We are very thankful to Dr. Sudhir Singh Scientist G (Entomological Division, Forest Research Institute, Dehradune, Uttarakhand) for identification of this

chrysomelid species *Perillus bioculatus* (Fab.) and Department of Zoology for providing required facilities and financial support by HNB Garhwal University.

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