



Original article

Does *Zygogramma bicolorata* Pallister really affects the growth, density and reproductive performance of *Parthenium hysterophorus* L?Fazil Hasan^{a,b,*}, Khalid A. Al-Ghanim^c, Fahad Al-Misned^c, Shahid Mahboob^c^a Department of Plant Protection, Faculty of Agricultural Sciences, Aligarh Muslim University, Aligarh 202002, India^b Division of Entomology, ICAR-Indian Agricultural Research Institute, New Delhi 110012, India^c Department of Zoology, College of Science, King Saud University, Riyadh 11451, Saudi Arabia

ARTICLE INFO

Article history:

Received 23 December 2019

Revised 13 March 2020

Accepted 22 March 2020

Available online 30 March 2020

Keywords:

Parthenium weed

Biological control

Population growth

Zygogramma bicolorata

Defoliation

ABSTRACT

Zygogramma bicolorata Pallister (Coleoptera: Chrysomelidae) is a satisfactory potential candidate for controlling *Parthenium hysterophorus* L. The study was conducted in order to evaluate the bio-control potential of *Z. bicolorata* to satisfactory reduced the density of parthenium weed in natural field conditions. Therefore, beetles were released in the twelve study sites @ 12 adults and/or larvae/m² in their respective sites on 5th, 10th, 15th and 20th day after initial field releases. Observations were made on defoliation by the beetle, parthenium plant height, plant biomass, leaf injury rating and soil seed reservoir. Present study showed that field releases of *Z. bicolorata* considerably lowering down the parthenium density at all treated sites. At larval release sites by the 60th day of observation a complete defoliation was noted, however at adult release sites it took 80 days for complete defoliation. Moreover, significantly higher number of developmental stages i.e. eggs, larvae and adults were noted in the adults released sites than to larval released sites and the controls. It was also noted that plant height and its density, flowers & leaves production, biomass of parthenium plant and seed reservoir in soil was negatively affected in treated sites as compared to untreated sites. Thus, we have concluded that adults can be utilized in biocontrol program because population build-up was greater in those sites where adults were released.

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1. Introduction

Congress grass, *Parthenium hysterophorus* L. (Asteraceae: Heliantheae) is herbaceous weed of international importance and originate from central and south America (Dolai et al., 2019). It is now considered as the most devastating weed of both agricultural and urban areas of Asia (Patel, 2011; Hasan and Ansari, 2016a) and Australia (Navie et al., 1996; Dhileepan and Strathie, 2009). After its introduction in late 1950's in Pune (Rao, 1956), it spread over an area of ~35 million ha of Indian lands including roadsides, railway tracks, cultivated fields and water lands (Singh et al., 2008). Parthenium weed spread to the large area very quickly due to its

high reproductive capacity (5000–10,000 seeds/plant), its light seed weight which disperse by wind very easily and lack of seed dormancy which allowed parthenium to complete at least 3–4 generations/year (Shabbir and Bajwa, 2006; Sushilkumar and Varshney, 2007; Sushilkumar and Ray, 2011; Hasan and Ansari, 2016b). Nevertheless, this weed is a responsible factor for causing many health related issues to human and livestock which have now reached to epidemic proportions (Mahadevappa, 1999; Singh, 2001; Sushilkumar, 2005; Patel, 2011). Warm temperature with sufficient rainfall favors the germination and growth of parthenium at any time of the year (Hasan, 2015; Hasan and Ansari, 2015). Effective management of parthenium is very difficult due to its capacity to produced seeds in large quantity, adaptability to grow well/flourish in any kind of habitats and allelopathic potential (Sushilkumar and Saraswat, 1999; Patel, 2011).

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 (Jayanth, 1987). After successful introduction, *Z. bicolorata* became colonized within 3 years and caused a considerable damage to the parthenium plants and lowering down its density in the trialed areas (Jayanth and Bali, 1994a; Sushilkumar and

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Ray, 2011). Nonetheless, in Jammu and Kashmir in 1992, adults of *Z. bicolorata* were mass released in parthenium prone areas at large scale (Gupta et al., 2002). After introduction, *Z. bicolorata* spread over >9000 km² area (Gupta, 2008; Gupta et al., 2010) and caused suppression of parthenium in many parts of the state (Gupta et al., 2004). Furthermore, Geographical Information System (GIS) based map was used by Dhileepan and Senaratne (2009) in order to adequately determine the current geographical distribution of *Z. bicolorata* in different states of India, as well as in neighboring countries.

Parthenium weed contains main allelochemical such as parthenin, which is known to cause a germination and radicle growth inhibitor in a variety of agricultural important economic crops. Nonetheless, this allelochemical inhibit shoot/root growth, nutrient uptake from soil and caused detrimental effects on naturally occurring symbiotic organisms (Hassan et al., 2017). Therefore, to control parthenium weed herbicidal application is the only methods remain, which offered good but time being results (Sushilkumar, 2005). Further, it is already known that indiscriminate use of synthetic herbicides can pose a threat to environment, soil fauna, human & animal health and other parts of ecosystem (Hasan and Ansari, 2016a). Nevertheless, eco-toxicity and/or non-target toxicity of herbicides can disrupt the integrated weed management programs by killing the natural enemies associated with weeds. Thus, the associated problems with herbicides have led to change in management strategies with the focus on long-term solution of weed using classical biological control (Syrett et al., 1999; Hasan and Ansari, 2017). Therefore, we can reduce the usage of herbicides by field releasing of natural enemies time to time to control parthenium. Plethora of literature is available on population dynamics, dispersal, feeding and reproduction of *Z. bicolorata* (Jayanth and Bali, 1993a,b,c, 1994b; Jayanth et al., 1993, 1997; Hasan and Ansari, 2017). However, limited information is available on augmentation and colonization of *Z. bicolorata* for biological control of parthenium weed in India (Jayanth, 1987; Sushilkumar and Ray, 2011). Therefore, present study was needful in order to evaluate the impact of field release program of *Z. bicolorata* for biological suppression of parthenium in Aligarh region, India.

2. Materials and methods

2.1. Study areas

Field experiments were conducted in 12 locations. It is situated at latitude 27°53'N and longitude 78°05'E/27.88°N. These locations were selected on the basis of presence of lush green parthenium plants almost throughout the year and have not been utilized for cultivation of any crop for at least one and a half year prior to experiments. These selected locations have parthenium infestations with a density of 60–65 plants/m² and the average height ranged from 20 to 60 cm and moisture ~80 to 85%.

2.2. Culture maintenance in protected field conditions

A bulk culture of *Z. bicolorata* was maintained on parthenium plants grown in 30 plastic pots (18 cm diameter; 20 cm deep) kept inside insect-proof cages (80 × 85 × 55 cm; each pot in each cage), in the experimental field conditions of Department of Plant Protection, Faculty of Agricultural Sciences. These plastic pots were filled with sun solarized soil mix with a layer of coconut coir at the base of pots for appropriate drainage and proper maintenance of moisture required for the growth of parthenium plants. Same aged parthenium seedlings (~2 or 3) were transplanted in these pots and irrigated whenever required. Five pairs of adults were released on each potted plants (30 days old) for egg laying and develop-

ment. Adults and immature of this beetle were provided with new parthenium plant time to time when previous plants were defoliated. Moisture was maintained in pots for the better growth of parthenium as well as quick development of the beetles. Developmental stages i.e. adults of the same age and 3rd instars of *Z. bicolorata* were picked up time to time from their mass rearing cages and utilized in subsequent experimentations.

2.3. Field releases of *Z. bicolorata*

Field releases of both adults (same age) and 3rd instars of *Z. bicolorata* was made at 8 locations (4 locations for adults and 4 locations for larvae) @ of 12 individuals/m² and 4 locations were left as controls, thus a total of 12 locations were chosen for experimentation. The field release was made on 1st May 2010 and further releases were carried out on 5th, 10th, 15th and 20th day after initial release. Experimental sites used for control were remained as such without releasing of adults and larvae of *Z. bicolorata*.

2.4. Observations on released sites

2.4.1. Damage potential of *Z. bicolorata*

The observations were made from 20th to 80th days after initial inoculation at an interval of 20 days. Twenty-five parthenium plants were tagged randomly to examine the level of defoliation, in each location. In larval and adults released sites, the damage rating was designated from 0 to 100% by visually observing the plants. Locations designated as control were also examined for natural attack by this beetle. The plants with complete defoliation and no flower were designated as 100% damage. Each plant was considered as a replicate.

2.4.2. Population growth of *Z. bicolorata*

Population growth/build-up of *Z. bicolorata* was also accessed by manually observing the developmental stages (i.e. number of eggs, larvae and adults) on infested plants at each site after an interval of 20th days right from the first release until a complete defoliation is noted.

2.4.3. Impact of field releases of *Z. bicolorata* on plant height

Plant height was examined in randomly selected 25 plants from each study site and the shoot of these plants were measured just above the ground level to the terminal portion of the plants with the help of measuring tape. Measurements of plant height were taken in the second week of November (2010 and 2011) as most of the adults of *Z. bicolorata* undergone diapause and no chance of plant damage is remained.

2.4.4. Impact of field releases of *Z. bicolorata* on weed density

The density of the weed was only monitored in the month of July (2nd week) 2010, 2011 and 2012, by directly counting parthenium plants/m² at all experimental sites and replicated four times.

2.4.5. Impact of field releases of *Z. bicolorata* on flowers and leaves production

The impact of feeding on flowers and leaves was assessed by counting of the flowers and leaves of 20 tagged plants from each site in second week of May 2012.

2.4.6. Impact of field releases of *Z. bicolorata* on plant biomass

Twenty plants from each location were selected and uprooted just above the ground level in second week of May 2012 and allowed to dry in oven at 60 °C. The biomass of plants was measured using electronic balance (Precision balance, CB-Series, Con-tech) and each plant was considered as a replicate.

2.4.7. Impact of field releases of *Z. bicolorata* on soil seed storage

At the completion of the experiment (second week of July 2012), soil samples (2.5 cm depth) were collected randomly from five sites each in control, larvae and adults release locations. Collected soil was brought to the laboratory and spread in plastic trays (15 × 8 × 4 cm) already filled with sterilized garden soil. Sufficient moisture was provided using sprinkle bottle for proper germination of seeds of parthenium stored in soil collected from experimental sites. Thus, the number of sprouted seeds/m² was then counted.

2.5. Statistical analysis

Logistic regression analysis (Pearson- χ^2) was performed in proportional binomial data of per cent damage before one way analysis of variance (ANOVA). Data of plant height (cm) of two consecutive years (2010 and 2011) were compared by the two-way analysis of variance. All treatment means were compared by a post hoc Tukey's HSD test. Statistical analysis and graphic present

ation were performed in the language program R 2.10.1, (R Development Core Team 2010).

3. Results

3.1. Damage potential of *Z. bicolorata*

Zygogramma bicolorata have caused considerable damage to various plant parts i.e. leaves, stem and flower of *Parthenium* when they established in the field (Fig. 1). Box plots demonstrated that the larval released sites have caused a significant more damage than the adults released sites. The damage was increased with time as indicated by the 20th ($F = 35.87$; $df = 2, 74$; $P < 0.001$) and 40th ($F = 45.89$; $df = 2, 74$; $P < 0.001$) day after initial inoculation. Furthermore, it was noted that larvae have caused a complete damage by the 60th day ($F = 76.77$; $df = 2, 74$; $P < 0.001$). Whereas, 80 days ($F = 95.97$; $df = 2, 74$; $P < 0.001$), were required in complete damage to the plants in the adults released sites (Fig. 1). However, compared of treated and untreated sites at 80th days showed a significantly difference, and minor damage was evident in control sites.

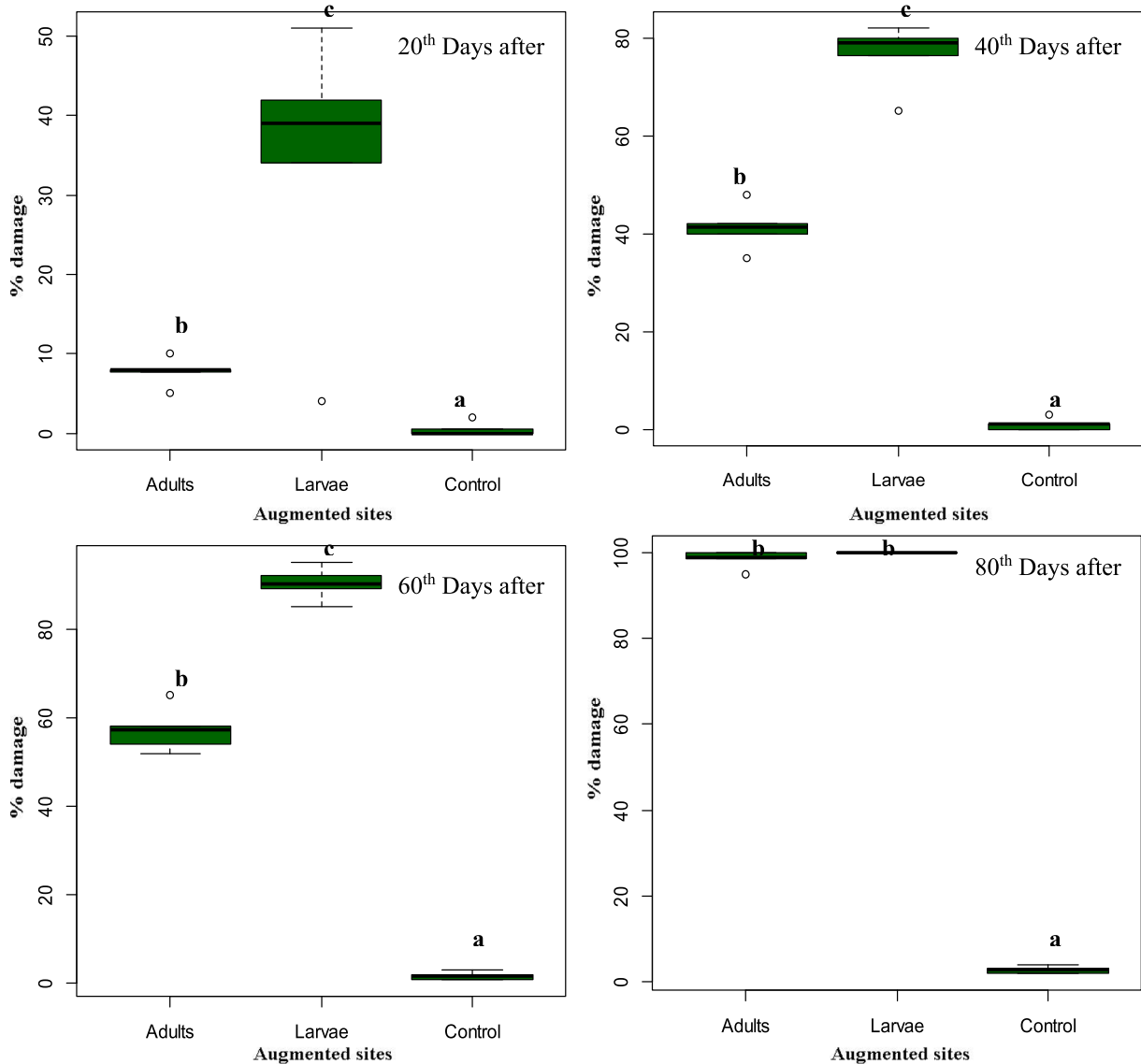


Fig. 1. Damage (%) at adults/larvae released and untreated sites. Box plots with similar letters are not significantly different (Tukey's HSD; $P > 0.001$).

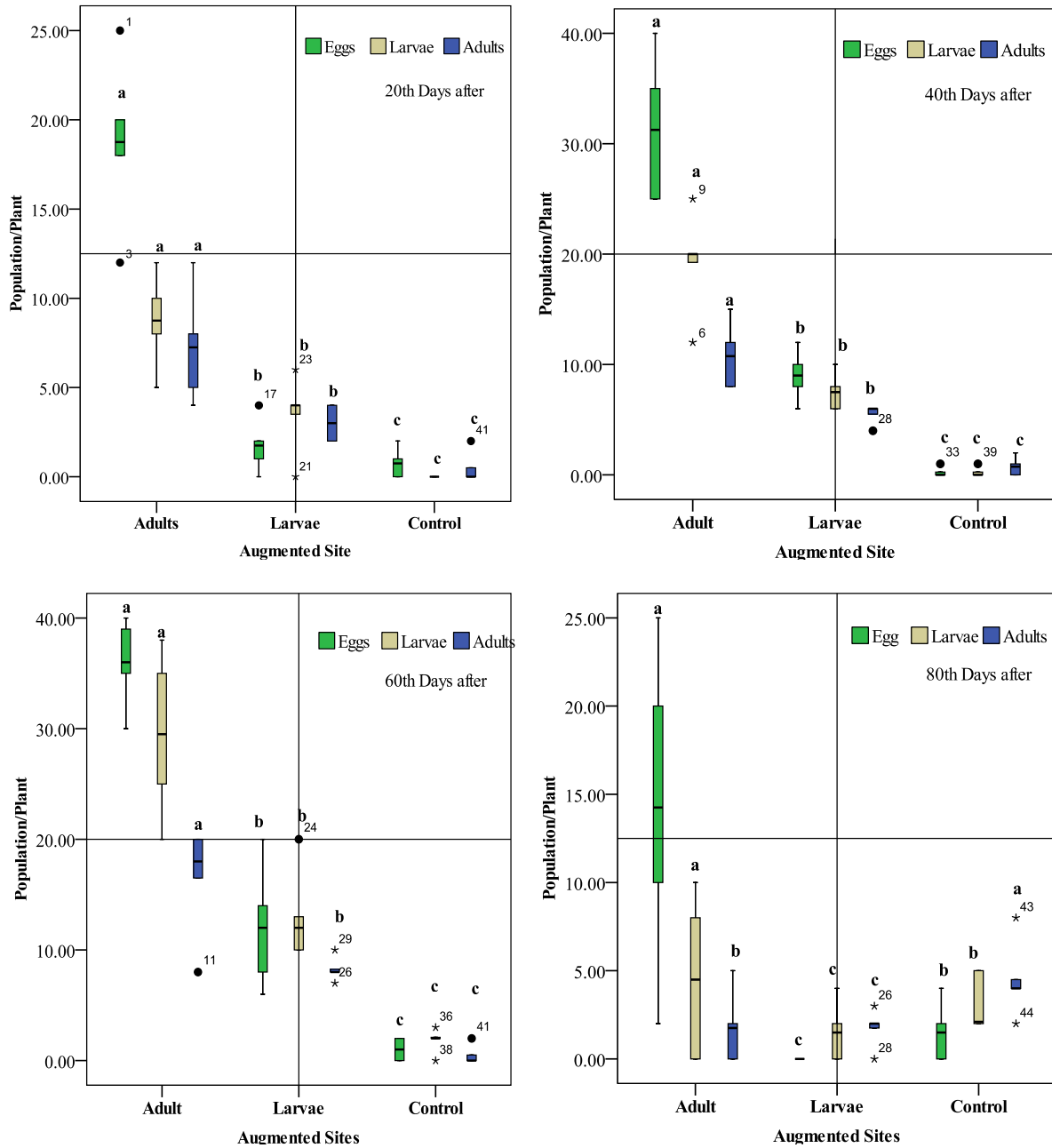


Fig. 2. Population/plant of *Z. bicolorata* in adults/larvae released and untreated sites. Box plots with similar letters are not significantly different (Tukey's HSD; $P > 0.001$).

3.2. Population build-up of *Z. bicolorata*

Results showed that population build-up was not quick in larval released sites as compared to adults released sites by the 20th day of observation ($F = 33.39$; $df = 2, 74$; $P < 0.001$). However, population of *Z. bicolorata* and its damage potential increased with time in larval released sites as indicated in 40th day ($F = 45.89$; $df = 2, 74$; $P < 0.001$) and 60th day ($F = 76.39$; $df = 2, 74$; $P < 0.001$) of inoculation (Fig. 2). But due to 100% defoliation to the plants in adults and larval released sites by 80th day, the population tends to negate ($F = 95.77$; $df = 2, 74$; $P < 0.001$). However, in control plots population of *Z. bicolorata* were also observed in 60th day of obser-

vation (mid July), though no individuals of this beetle were released (Fig. 2).

3.3. Impact on plant height

The observations on plant height in larval and adults released sites in 2010 indicated that there was no significant difference, however, the considerable difference was detected in height of the plants in the treated and untreated sites in both study years ($F = 0.25$; $df = 1$; $P = NS$ *Years, $F = 60.15$; $df = 2$; $P < 0.001$ *Treatments, $F = 4.45$; $df = 2$; $P < 0.015$ *Years \times Treatments at total $df = 149$). Furthermore, results of the present study indicated a sig-

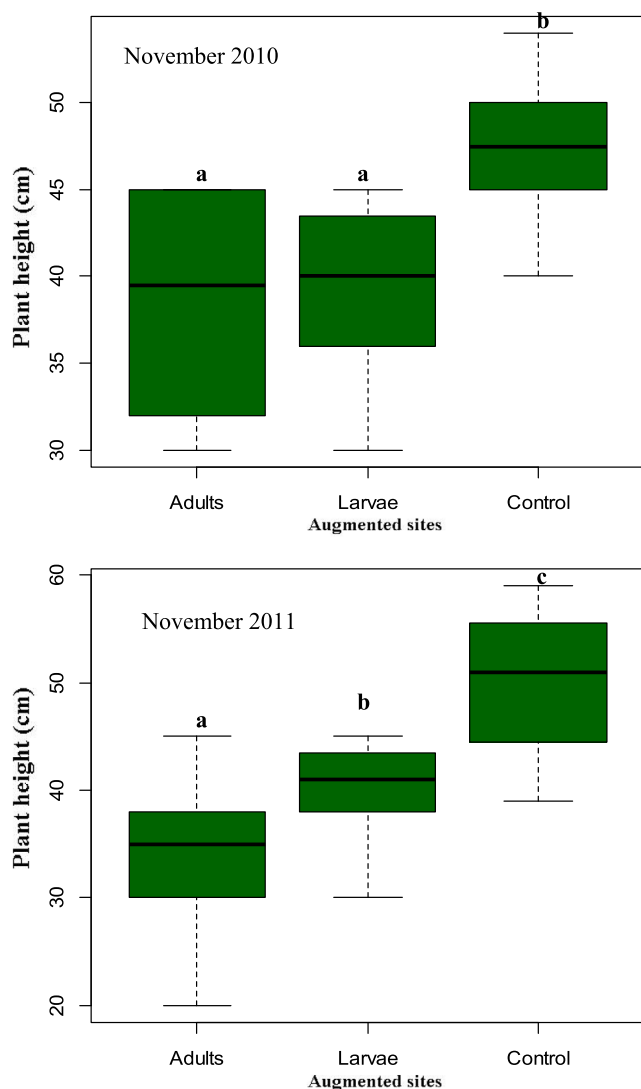


Fig 3. Impact of field releases of adults/larvae of *Z. bicolorata* on plant height of *P. hysterophorus* in 2nd week of November of the year 2010 and 2011. Box plots with similar letters are not significantly different (Tukey's HSD; $P > 0.001$).

nificant difference in the height of plants in adults and larval released sites in the year, 2011 (Fig. 3).

3.4. Impact on plant density

Plant density was also significantly affected by larvae and adults augmented sites from 2010 ($F = 0.37$; $df = 2, 11$; $P = NS$) to 2012 ($F = 112.37$; $df = 2, 11$; $P < 0.001$) as compared to untreated sites (Fig. 4).

3.5. Impact on flowers and leaves production

It was evident a lower rate of flowers ($F = 1320.46$; $df = 2, 59$; $P < 0.001$, Fig. 5) and leaves production ($F = 221.54$; $df = 2, 59$; $P < 0.001$, Fig. 6) at all treated sites. Further, larval released sites had lowest number of leaves as compared to adults released sites.

3.6. Impact on plant biomass

Plant biomass was significantly highest in control sites ($F = 561.95$; $df = 2, 59$; $P < 0.001$) followed by larval and adults augmented sites. Larval and adults augmentation in field resulted in decreased in plant biomass to 42.9% and 56.64%, respectively when compared to the biomass of plants grown in control plots (Fig. 7).

3.7. Impact on soil seed storage

Up to a regular damage by *Z. bicolorata* for more than 120 days have significantly reduced the reservoir of parthenium seeds in soil by 75% ($F = 1451.45$; $df = 2, 14$; $P < 0.001$) (Fig. 8).

Meteorological data on average temperatures, humidity and annual rainfall of years 2010, 2011 and 2012 of Aligarh region is presented in Fig. 9.

4. Discussion

This case study clearly demonstrated that the field releases of *Z. bicolorata* have negative impact on various fitness parameters i.e. leaves and flower production, soil seed reservoir, seed germination and adequate growth of parthenium plant. The outbreak of *Z. bicolorata* have caused 80–100% defoliation which resulted into considerable reduction of leaves and flower production, density of plants, plant growth, production of flowers and soil seed reservoir in almost all studied sites. In previous studies many workers

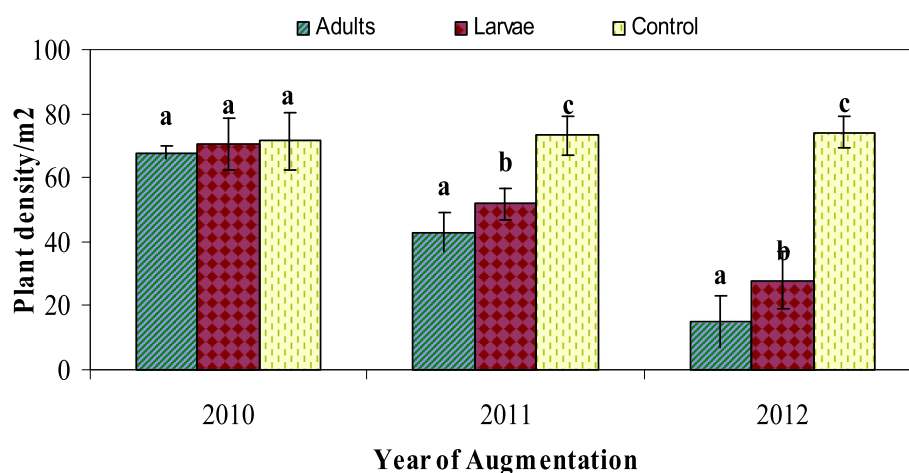


Fig. 4. Impact of field releases of *Zygogramma bicolorata* (adult and larvae) on parthenium plant density/ m^2 in year of 2010, 2011 and 2012. Bar plots with similar letters are not significantly different (Tukey's HSD; $P > 0.001$).

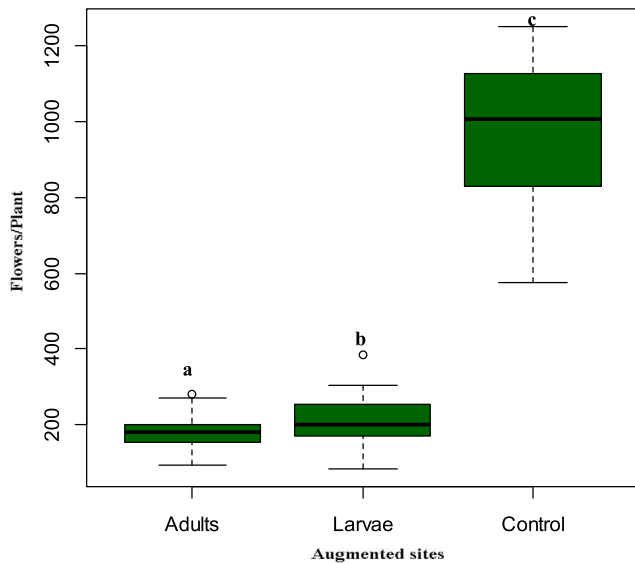


Fig. 5. Impact of field releases of *Zygogramma bicolorata* (adult and larvae) on flowers/plant of *P. hysterophorus* in 2nd week of May of the year 2012. Box plots with similar letters are not significantly different (Tukey's HSD; $P > 0.001$).

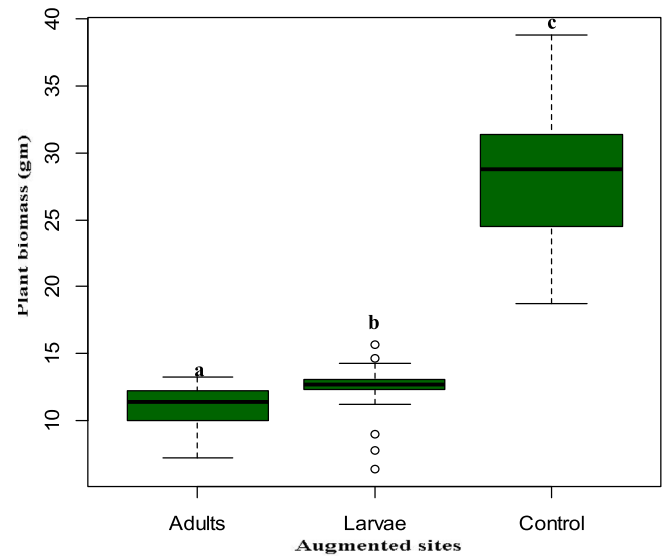


Fig. 7. Impact of field releases of *Zygogramma bicolorata* (adult and larvae) on plant biomass (gm) of *P. hysterophorus* in 2nd week of May of the year 2012. Box plots with similar letters are not significantly different (Tukey's HSD; $P > 0.001$).

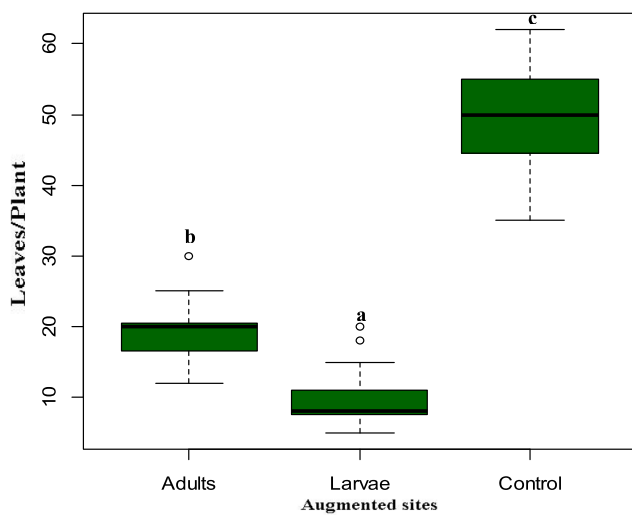


Fig. 6. Impact of field releases of *Zygogramma bicolorata* (adult and larvae) on leaves/plant of *P. hysterophorus* in 2nd week of May of the year 2012. Box plots with similar letters are not significantly different (Tukey's HSD; $P > 0.001$).

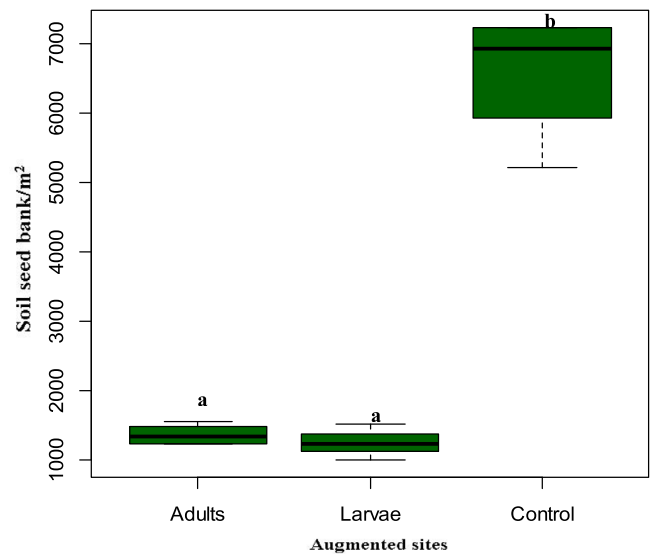


Fig. 8. Impact of field releases of *Zygogramma bicolorata* (adult and larvae) on soil seed reservoir/m² of *P. hysterophorus* in 2nd week of July of the year 2012. Box plots with similar letters are not significantly different (Tukey's HSD; $P > 0.001$).

have reported almost a similar percentage of defoliation (~85%) caused by *Z. bicolorata* and complete reduction in plant density in Indian agro climatic conditions (Jayanth and Bali, 1994a; Jayanth and Visalakshy, 1996; Sushilkumar and Ray, 2011). Nevertheless, McFadyen and McClay (1981) concluded that the defoliation and damage of parthenium plants by *Z. bicolorata* depends on geographic locations as well as study years under Australian scenario. Further, it was previously noted that after field releases of *Z. bicolorata* in the area of released sites can takes up to four years to get their colony well established and strong, necessary for the desirable biological control of parthenium weed (Jayanth, 1987). However, in many instances after well enough colonization of *Z. bicolorata* in the field the adequate biological control of this weed was not satisfactory due to slow growth, late emergence from diapausing state, slow feeding, high mortality rate and low reproduction of the beetles (Sushilkumar and Ray, 2011). Among these factors, diapausing behavior of this beetle considerably slow

down its biocontrol potential especially in the month of December where majority of the adult's population remains in diapause (Jayanth and Bali, 1993b; Hasan et al., 2017).

Our results showed that, the considerably highest number of developmental stages of *Z. bicolorata* i.e. eggs, larvae and adults were recorded in adults released sites which might be due to that after being released, female adult starts laying eggs and build-up of population. Therefore, laboratory reared adults if releases in field conditions on parthenium prone areas time to time, an extensive impact on weed density, its flower production and soil seed reservoir can be achieved. Defoliation by *Z. bicolorata* significantly reduced not only the plant height but also the flower and seed production. It might be due to the regular herbivory by this beetle which destroyed the growth pattern of apical meristem resulted into reduction in plant height, alteration in branching pattern and ultimately less or no production of flower and seeds

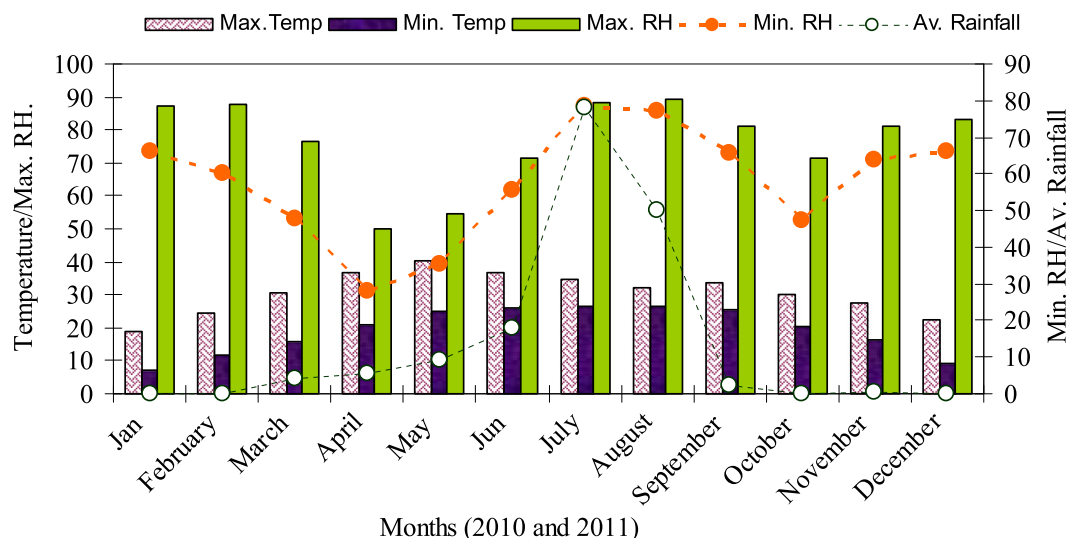


Fig. 9. Meteorological data of Aligarh region, UP, India, for the period of 2010 to 2012, illustrating mean monthly maximum and minimum Temperature, maximum and minimum relative humidity and average rainfall.

(Dhileepan et al., 2000). Previously, Kovalev and Medvedev (1983) reported that the herbivory in Ambrosia weed by the introduction of a related species i.e. *Z. saturalis* also caused a reduction of plant height, flower and seed production.

Nonetheless, results of this study demonstrated that in completely defoliated plants, *Z. bicolorata* targeted flower buds for egg laying. After hatching neonate feeds on flowers and by doing so preventing the seed production. Similarly, Dhileepan et al. (2000) have reported that 98% defoliation of parthenium plant do not produced any flower and seed. Furthermore, our results also showed that due to complete defoliation of plant and its density there was ultimately a reduction of number of flower and seed production/m² up-to 5 to 150-folds. Similarly, these observations were previously taken by Lonsdale et al. (1995) in *Sida acuta* and by Blosssey (1992) in *Lythrum salicaria* L. due to the introduction of their respective bio-control agents. This continuous defoliation of parthenium plant by *Z. bicolorata* upto 3 or more years significantly reduced the soil seed reservoirs which ultimately lower down the abundance of parthenium density in study locations (Dhileepan et al., 1996; Hasan and Ansari, 2015).

Moreover, for an effective biological control of parthenium a proper population build-up of *Z. bicolorata* is important, which could be attributed for timely releases of these beetles in the field. Nevertheless, field releases of the correct stage of this beetle are also as important as the correct time. Therefore, field releases of adult stage of *Z. bicolorata* are quite effective as they are responsible for build-up of whole population in less time, thereby decreasing the density of parthenium plant. Our study showed that field releases of adults of *Z. bicolorata* considerably reduced the density of plant, affects the various growth stages, defoliated the plant, damage the flowers and lowering down the rate of seed formation (Sushilkumar and Ray, 2011). Similarly, workers have previously reported up to 100% defoliation of parthenium plant by *Z. bicolorata* (Hasan and Ansari, 2015; Hasan et al., 2017).

It could be concluded from the present investigations that larvae are effective defoliator than the adults but field releases of adults could provide effective management of parthenium plants. Because adults are responsible for population build-up and propagate the new colony of better performing individuals which leading to quick and effective control of parthenium at release site.

Declaration of Competing Interest

The authors declared that there is no conflict of interest.

Acknowledgments

F.H. is highly thankful to UGC, New Delhi, India for financial assistance during this research program. F. H is thankful to Prof. Athar Ali Khan, D/O Statistics and Operation Research, Aligarh Muslim University, Aligarh, India for his statistical suggestions and graphical presentation in this manuscript. The authors (SM and KAAG) express their sincere appreciation to the Deanship of Scientific Research at the King Saud University for its funding of this research through the Research Group Project No. RG-1435-012.

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