Indian Journal of Weed Science (2022) **54**(1): 58–65
http://dx.doi.org/10.5958/0974-8164.2022.00010.7

RESEARCH ARTICLE

Enhanced biological control of Parthenium by release of female dominated sex ratio population of *Zygogramma bicolorata* Pallister

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Received: 3 March 2022 | Revised: 26 March 2022 | Accepted: 27 March 2022

ABSTRACT

Parthenium hysterophorus L. (Asteraceae) is a major weed in both cropped and non-crooped areas of India and many other countries. Considering the magnitude of problems caused by *P. hysterophorus*, its management is essential to prevent future complications. Leaf-feeding beetle *Zygogramma bicolorata* Pallister (Coleoptera: Chrysomelidae) has been proved as the most promising biocontrol agent of Parthenium in India and other countries. In the present study, sex ratio and sexual dimorphism of *Z. bicolorata* was studied using field samples collected during September month of 2013 to 2015 from different sites at Jabalpur (Madhya Pradesh, India). The male and female average sex ratio was observed as 1:1.50, 1:1.61 and 1:1.46 in 2013, 2014 and 2015, respectively. Sex ratio was significantly deviated towards the female. Females were distinctly larger and heavier in body size and abdominal width than the males. Further, experiments were conducted during rainy season of 2015 and 2016 to find out the effect of female dominated sex ratio, body weight and size by releasing of 7500 beetles/ha in two sex ratio *viz.* 1: 1 and 1: 1.60. Significant difference was recorded amongst these two sex ratios on density, height of plants, dry weight and number of flowers in Parthenium weed at 30 and 60 days after release of bioagent. Significantly higher effect of female dominated sex ratio (1:1.60) release was found in suppression of Parthenium. Therefore, for better and assured control, female dominated releases were recommended under biological control programme of Parthenium.

Keywords: Biological control**,** Biocontrol efficiency, Body weight, Body size, Sex ratio, Sexual dimorphism, Parthenium, *Zygogramma bicolorata*

INTRODUCTION

India has become one of the most Parthenium affected country in the world where about 35 million hectares of land is estimated to be affected by Parthenium (Sushilkumar and Varsheny 2010). *Zygogramma bicolorata* Pallister (Coleoptera: Chrysomelidae), a native of Mexico is a proven biological control agent of Parthenium in Australia **(**McFadyen 1992, Dhileepan 2003, Adkin *et al.* 2014) and India (Sushilkumar 2009, 2014). The bioagent has been released in various parts of the world suffering from Parthenium (Dhileepan and Senaratne 2009, Adkins and Shabbir 2014) invasion including India (Sushilkumar 2014).

The sexual dimorphism refers to differences between females and males of a species in terms of behavior, size and appearance. Male and female size affects production of progeny (Emlen and Oring 1977) and also influence mating behavior of insect species (Keller *et al.* 2011). Small size males may be more vulnerable to pressures than larger females while larger females are supposed to produce more eggs having more survival efficacy (Dhiman and Bhargava 2005, Pawar *et al.* 2015).

Sex ratio is one of the most important factors for insect biocontrol agent population establishment and subsequent impact on host in the natural environment where population is increased and established under abiotic and biotic factors (Omkar *et al*. 2013, Hopwood *et al*. 2016). Sex ratio affects population density and dynamics and future predictions for population establishment in natural conditions (Benowitz *et al*. 2013, Lachowsky and Reid 2014, Smith and Belk 2018). Hasan and Ansari (2016) reported that the proportion of male in a mixed population of adults showed greater numbers of female occurrence in future progeny. Sex ratio plays a significant role for species survival in any bisexually breeding population apart from biotic and abiotic factors. Knowledge on sex ratio of a weed bioagent species may help to understand its competence to bring destruction of a weed in a biological control program. Population dynamics is dependent on sex-ratio which influence population growth rates in response to the conditions **(**Southwood and Henderson 2000) and also helpful to

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understand growth and survival of an insect population (Wittmeyer and Coudron 2001).

The research work on *Z. bicolorata* focused, till to date, mainly on its occurrence, spread, and general biology including sex ratio and reproductive behavior besides general effect on Parthenium management after release of the bioagent (Jayanth and Visalakshy 1994, Sushilkumar 2009, Dhileepan *et al*. 2000). But, quantitative data is unavailable on its sex ratio through big size sample collection from fields and impact of release of different sex-ratio population in suppression of Parthenium in context to different biological parameters. In this study, our aim was to quantify the dominance of sex and impact of release of different sex ratio population on their efficiency in the management of Parthenium under the biological control program.

MATERIALS AND METHODS

Collection of beetles

The study was carried out during 2013 to 2015 at ICAR-Directorate of Weed Research (DWR), Jabalpur (Madhya Pradesh). The center is located between 22.49 and 24.8 North latitude, 78.21 and 80.58 East longitude and at an altitude of 412 meters above the mean sea level. Jabalpur comes under the agro-climatic region of Kymore plateau and Satpura hills and lies in the rice-wheat crop zone of the state. The climate of Jabalpur region is typically sub-humid and sub-tropical. Adult beetles were collected by hand from 10 different locations of Jabalpur in the first week of September (most active period of beetle) each year and were brought to the laboratory. Collected *Z. bicolorata* beetles were kept in the laboratory in wire mesh cages with window arrangement to enable us to catch the paired beetles from inside. The fresh Parthenium twigs with leaves were provided for food and perching.

Sex ratio study

In the laboratory, paired beetles in copulation were picked up from cages and sexes were separated as male and female by marking the male mounted over female. Left beetles which were not found paired were identified based on last abdominal sternite as described by McClay (1980). The males and females from different sites in different years were counted and the percentage of male and female were calculated. Sex ratio was analyzed as the proportion of offspring that are males and calculated according to Wilson & Hardy (2002) with the help of formula given below:

$$\text{Sex ratio} = ♂♂/(♀♀ + ♂♂)$$

Sexual dimorphism

A biometric study for sexual dimorphism was made for 50 individuals of each sex collected during 2015. The body parts namely, antennal length, total body length from head to end of abdomen with elytra, maximum width of abdomen with elytra and head-width with eyes were measured under the binocular microscope (Leica make, model No.WILD M3Z).

Weight and moisture content estimation

For body weight and moisture content study, freshly collected adults after anesthetizing were weighted individually (30 for each sex) in electronic balance (Danwer Scales (India) Pvt. Ltd., Model-Dw302) for fresh net weight. After taking fresh weight, these were dried in vacuum oven at 60 ^{0}C and weighted for dry weight and thus calculated moisture content.

Biocontrol efficiency of bioagent *Z. bicolorata* under equal and female dominated ratio release

The seeds of Parthenium weed, collected during March-April 2015 and 2016, were sown on 20 June 2015 and 15 June 2016, respectively by broadcasting method in three plots each having an area of 0.21 hectare at a distance of about 500 meters from each other. Bioagent *Z. bicolorata* was released in male female ratio of 1:1 and 1:1.6 at the rate of 7500 beetle per hectare in each plot in each year after 30 days of sowing when Parthenium weed grew up to an average of 15±1.30 cm size. The same size area was left untreated (control) at the distance of 500 meter from the treated area. To nullify the effect of stray bioagent in untreated area, spray of insecticide imidacloprid 2 ml/l was done at monthly interval. Density of Parthenium was taken at randomly from 20 places from each bioagent released plots and control plots with the help of 1 m^2 iron quadrat at 60- and 90-days interval after sowing of Parthenium (30 and 60 days after release of beetle). From each quadrat, one plant was sampled at random for total no. of eggs, grubs and adults, height of plants, flowers per plant and dry weight (biomass). Impact of beetles on Parthenium was calculated in terms of reduction in plant density, height, number of flowers and dry weight. Bioagent control efficiency was calculated as per formulae given below:

$$\text{Biocontrol efficiency} = \frac{\text{Dry weight of control plot} - \text{Dry weight of treated plot}}{\text{Dry weight of control plot}} \times 100$$

Statistical analysis

Equality of error variances were checked using F-test for two sample means. Three factor asymmetrical design was adopted to analyze data on the number of male and female in different years observed in 10 sites. These were subjected to ANOVA for comparing means using least significant difference (LSD) value. Statistical analysis was done using SAS 9.3 (SAS Institute *Inc*., USA). Chi square test was applied to the data of male and female count to check whether beetle population carries the male and female proportion in the ratio of 50:50.

RESULTS AND DISCUSSION

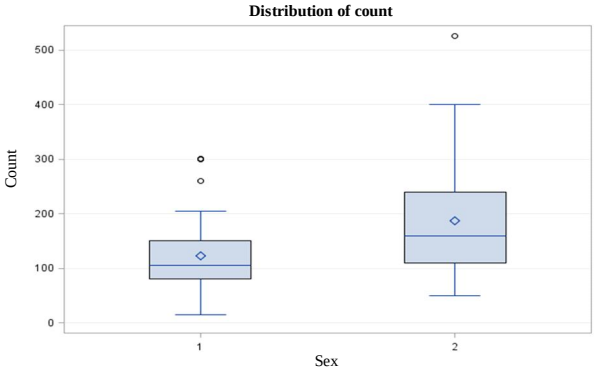
A total of 9296 adult beetles were captured, comprising 2140, 2485 and 4671 during 1st week of September of 2013, 2014 and 2015, respectively from 10 fixed sites at different locations of Jabalpur in each year.

Sex ratio

Sex-ratio was obtained as the proportion of male beetles to the total beetles. A highly significant difference was found between number of males and females. The observations on distribution of male and female population in three years indicated that in all places/sites, female population dominated irrespective of the years (F=10.22; df=1, 54; P=0.01) (**Figure 1**). Hasan and Ansari (2016) also found that the female population was significantly higher over the male population in each month of years in their study.

Male and female *Z. bicolorata* beetles frequencies in different years as well as in different sites have been given in **Table 1** along with their chi-square values and significance level.

The average male and female populations of three years were 120.7 and 182.7, respectively. The



1-male, 2-female (F=10.22; df=1, 54; p=0.01)

Figure 1. The *Z. bicolorata* male and female population average distribution across three years

percentage of sex ratio varied with each sample. There was male dominance only at sites No. 3 and 4 during 2013; site No. 1, 2 and 3 during 2014 and site no. 9 during 2015, but pooled averaged data revealed the significant dominance of females (chi-square $\div^2$ = 7.9, 13.8, 16.7 (for 2013, 2014, 2015 respectively); df = 1; P=0.01). The level of sex ratio in *Z. bicolorata* varied significantly between years. Results **(Figure 1)** showed that sex ratio (proportion of male) was also greatly influenced each year ($p < 0.05$). Female population was significantly superior over the male population in all the studied years and sex ratio variation was statistically significant.

Sexual dimorphism and correlation of *Z. bicolorata* body parts

Total average length of male and female *Z. bicolorata* beetles was 5.46±0.50 and 6.48±0.50 mm, respectively. Simultaneously, average abdomen width of male and female beetles was 2.74 ± 0.44 and 3.66 ± 0.47 mm, respectively. Females were distinctly

Table 1. The variation in male and female population of *Z. bicolorata* across different samples and years

Sample no.	Year	*Z. bicolorata* population				Chi-square value with 1 d.f.	Significance level
		Male	Female	Total	Male: Female ratio		
1	2013	80	90	170	1:1.13	0.59	NS
	2014	130	120	250	1:0.92	0.40	NS
	2015	200	286	486	1:1.43	15.2	**
2	2013	110	160	270	1:1.45	9.3	**
	2014	110	60	170	1:0.55	14.7	**
	2015	58	160	218	1:2.76	47.7	**
3	2013	101	80	181	1:0.79	2.4	NS
	2014	120	80	200	1:67	8.0	**
	2015	301	526	827	1:1.75	61.2	**
4	2013	68	50	118	1:0.74	2.7	NS
	2014	100	110	210	1:1.10	0.5	NS
	2015	165	250	415	1:1.52	17.4	**
5	2013	130	225	355	1:1.73	25.4	**
	2014	100	400	500	1:4.00	180.0	**
	2015	300	380	680	1:1.27	9.4	**
6	2013	55	166	221	1:3.02	55.8	**
	2014	100	200	300	1:2.0	33.3	**
	2015	80	185	265	1:2.31	41.6	**
7	2013	20	135	155	1:6.75	85.3	**
	2014	15	95	110	1:6.33	58.2	**
	2015	200	345	545	1:1.73	38.6	**
8	2013	150	170	320	1:1.13	1.3	NS
	2014	50	130	180	1:2.60	35.6	**
	2015	125	145	270	1:1.16	1.5	NS
9	2013	85	110	195	1:1.29	3.2	NS
	2014	125	180	305	1:1.44	9.9	**
	2015	260	240	500	1:0.92	0.8	NS
10	2013	55	100	155	1:1.82	13.1	**
	2014	100	160	260	1:1.60	13.8	**
	2015	205	260	465	1:1.27	6.5	*
Mean	2013	77.64	116.90	467.1	1:1.51	7.9	**
	2014	95	153.5	248.5	1:1.61	13.8	**
	2015	189.4	277.7	467.1	1:1.47	16.7	**

* and ** denote the significant values at 5% and 1% level of significance, respectively

larger both in body size and abdominal width. Average antennal length and head width of male and female beetles were 2.58± 0.49, 2.50 ± 0.50 and 2.68 ± 0.47, 2.68 ± 0.47 mm, respectively. Adult beetles' body parts were correlated with head, antenna, abdomen and body length. The width of head and abdomen also increased with length of antenna and body length. Females were distinctly larger both in body-size and abdominal width while antenna was not significantly different in length but female head was larger than male **(Figure 2).** No significant difference was found between the number of antennal segments of male and female beetles of *Z. bicolorata*. The posterior margin of the female was entirely blunt, while it was slightly serrated at the tip in case of male.

Average fresh weight of male and female *Z. bicolorata* beetles was recorded 2.19±0.08 and 2.89±0.02 mg/beetle and dry weight as 1.29 ±0.20 and 1.82± 0.12 mg/beetle, respectively. Average percentage of moisture content of male and females was 58.9±10.0 and 63.2± 4.19, respectively (**Figure 3**). There was considerable variation in the wet and dry weights of *Z. bicolorata*. The females were distinctly heavier than the males ($p<0.001$) both in wet and dry weights and moisture content ($p<0.001$).

The density, biomass and flower production of Parthenium at 30 and 60 days after release of *Z. bicolorata* adult population in different male and female ratio

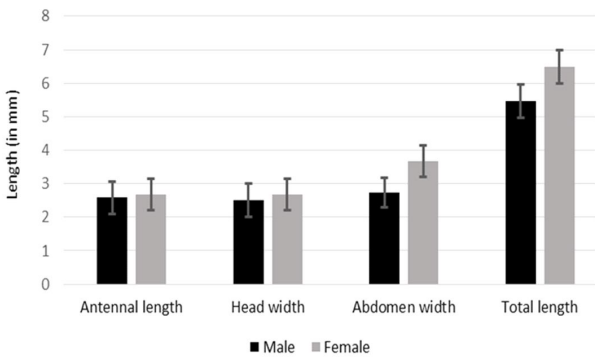
Two treatments with *Z. bicolorata* beetles and without beetles were compared in two different time intervals at 30 and 60 days after release (DAR) of bioagent and with different ratios (1:1 and 1:1.6). Initially at 60 DAR, beetle in 1:1 ratio could able to reduce the Parthenium density, biomass and plant height significantly as compared with the control plots, where beetles were not released (= 38.86, 25.42, 18.25, 53.26 for density, dry weight, plant height and flowers, respectively df = 38; p=0.01) **(Table 2)** while on the other hand, beetles in 1:1 ratio were unable to reduce these parameters in the Parthenium plants at 30 DAR. The beetles released in male: female ratio of 1:1.6 reduced the plant height and biomass significantly, but did not reduce the Parthenium flowers number significantly at 30 DAR, but at 60 DAR, they immensely reduced the Parthenium density, biomass, height and number of flowers (t = 16.79, 18.13, 11.96, 32.31 for density, dry weight, plant height and flowers, respectively; df = 38; p=0.01).

Interaction was also significant at 5% level of significance. It can be seen that beetles in 1:1.60 ratio reduced the density, dry weight, height and flower of Parthenium significantly at both 30 and 60 DAS. Greatest reduction in these parameters were obtained at 60 DAS with 1:1.60 ratio (**Table 3**).

Effect on Parthenium flower production

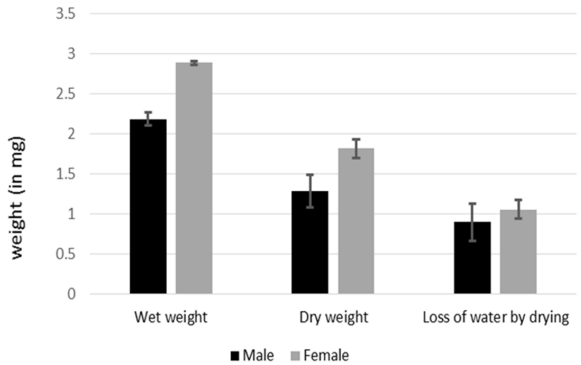
The 1:1.6 male: female ratio of *Z. bicolorata* beetles gave good control of Parthenium with the progress in time, leaving very few flowers to produce seeds and subsequently caused complete damage to plants **(Figure 4)**. Thus, under females dominated ratio (1:1.6) in a defined area, greater suppression of Parthenium weed occurred compared to the 1:1 ratio of male and female. Release of the adult population in 1:1.6 ratio, reduced the flowers at 30 DAR to the extent, which was reduced by release of 1:1 ratio at 60 DAR. Same pattern was also followed in the case of density and biomass of Parthenium.

Analysis revealed significant difference between heights of the plants at 30 and 60 days after release in treated plots (F =126.9; df =1, 76; P=0.0001) with mean values as 44.6 and 34.35 cm, respectively in 1:1 and 1:1.60 ratio of male and female at the rate of 7500 beetles/ha and also showed significant difference on the height of the plants (F=



Error bars shows the standard deviation of the data

Figure 2. Sexual dimorphism between male and female of *Z. bicolorata* beetle



Error bars are the standard deviation among the data points

Figure 3. Variation in weight and moisture content between male and female *Z. bicolorata*

Table 2. Effect of *Z. bicolorata* on Parthenium density, biomass, height and flower number suppression of Parthenium at 30 and 60 days of release (DAR) in comparison to non-release (control) of bioagent

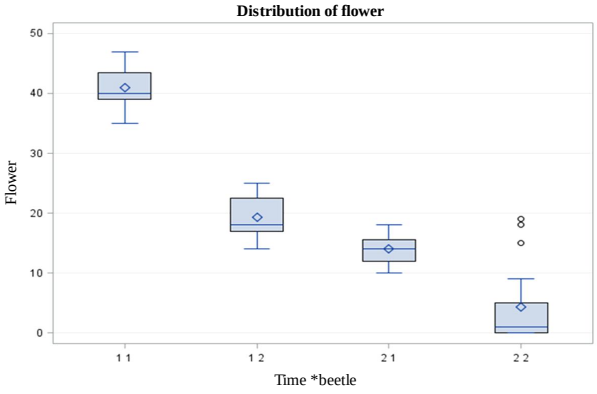
Treatment		Parthenium			
		Density (no./m^2)	Biomass (g/plant)	Height (cm/plant)	Flower (no./plant)
30 DAR	With bioagent (male: female (1:1)	352.1	7.20	31.10	19.35
	Without bioagent	335.8	8.55	33.5	19.30
	|t| value	0.75 (NS)	1.77 (NS)	1.11 (NS)	0.02 (NS)
60 DAR	With beetles (male: female (1:1)	60.8	5.65	49.05	14.05
	Without beetles	159.2	26.15	102.6	363.4
	|t| value	38.86 (S)	25.42 (S)	18.25 (S)	53.26 (S)
30 DAR	With beetles (male: female (1:1.6)	334.5	8.65	37.6	40.95
	Without beetles	389.8	14.65	44.7	45.40
	|t| value	7.69 (S)	23.35 (S)	6.36 (S)	1.27 (NS)
60 DAR	With beetles (male: female (1:1.6)	21.45	1.05	40.15	4.3
	Without beetles	93.35	26.95	103.2	395.7
	|t| value	16.79 (S)	18.13 (S)	11.96 (S)	32.31 (S)

(Significance level is given in parentheses. S-Significant at 1% level of significance, NS-Not significant, DAR-Days after release of beetle)

Table 3. Separate and interactive effect of bioagent *Z. bicolorata* released at different ratios on Parthenium density, biomass, height and flowers numbers

Treatment	Parthenium			
	Density (no./m^2)	Biomass (g/plant)	Height (cm/plant)	Flowers (no./plant)
Time				
30 DAR	343.25±12.44^a	7.93±1.02^a	34.4±4.6^b	30.15±15.27^a
60 DAR	41.13±27.82^b	3.35±3.25^b	44.6±6.29^a	9.17±6.89^b
LSD (p=0.05)	6.81	0.48	1.82	1.82
Release of beetles in ratio (Male: Female)				
1: 1	197.63±93.49^a	7.15±2.12^a	43.3±8.09^a	27.5±19.02^a
1.1.60	186.75±133.77^b	4.13±3.34^b	35.6±6.4^b	11.8±10.64^b
LSD (p=0.05)	6.81	0.48	1.82	1.82
Time× ratio release				
30 DAR×1:1	334.45±21.60^b	8.65±0.48^a	37.60±3.37^c	41.0±3.20^a
30 DAR×1.1.60	352.05±19.31^a	7.20±1.06^b	31.1±2.92^d	19.4±3.76^b
60 DAR×1:1	60.80±6.50^c	5.65±1.08^c	49.1±5.58^a	14.1±1.95^c
60 DAR×1.1.60	21.45±7.29^d	1.05±1.46^d	40.2±3.9^b	4.3±6.2^d
LSD (p=0.05)	9.25	0.72±	2.58	2.34

Different superscripted letters show significant difference in the treatment



1 1 - 30 DAR×1:1, 1 2 - 30 DAS×1:1.6, 2 1 - 60 DAS×1:1; 2 2 - 60 DAS×1:1.6

Figure 4. Number of Parthenium flowers at 30 and 60 days after release (DAR) as influenced by male: female ratios of released beetles' population (F = 126.9; df = 1, 76; P=0.0001), (F = 71.6; df = 1, 76; P=0.0001), (F = 126.9; df = 1, 76; p=0.0001)

1, 76; p=0.0001), (F = 71.6; df = 1, 76; p=0.0001), (F = 126.9; df = 1, 76; p=0.0001) 71.6; df = 1, 76; P=0.0001). Significant effect of number of beetles was observed on the flowers of the plants at different time intervals *i.e.* 30 and 60 DAR as their interaction was significant at 5% level of significance (F = 126.9; df = 1, 76; P=0.0001). Highly significant difference was observed between number of flowers on 30 and 60 DAR with varying number of females' beetles.

The distribution of different stages of *Z. bicolorata* namely eggs, grubs and adults on Parthenium plant showed significant variation during observation against 30 and 60 days after release (DAR) of bioagent. The release of beetles at ratio of 1:1, resulted in 32.95 eggs/plant, 9.1 grubs/plant, 3.45 adults/plant and 40.72 male ratio over female at 30 DAR while 44.27 female ratio over male. On the other hand, the release ratio of 1:1.6, resulted in 70.4

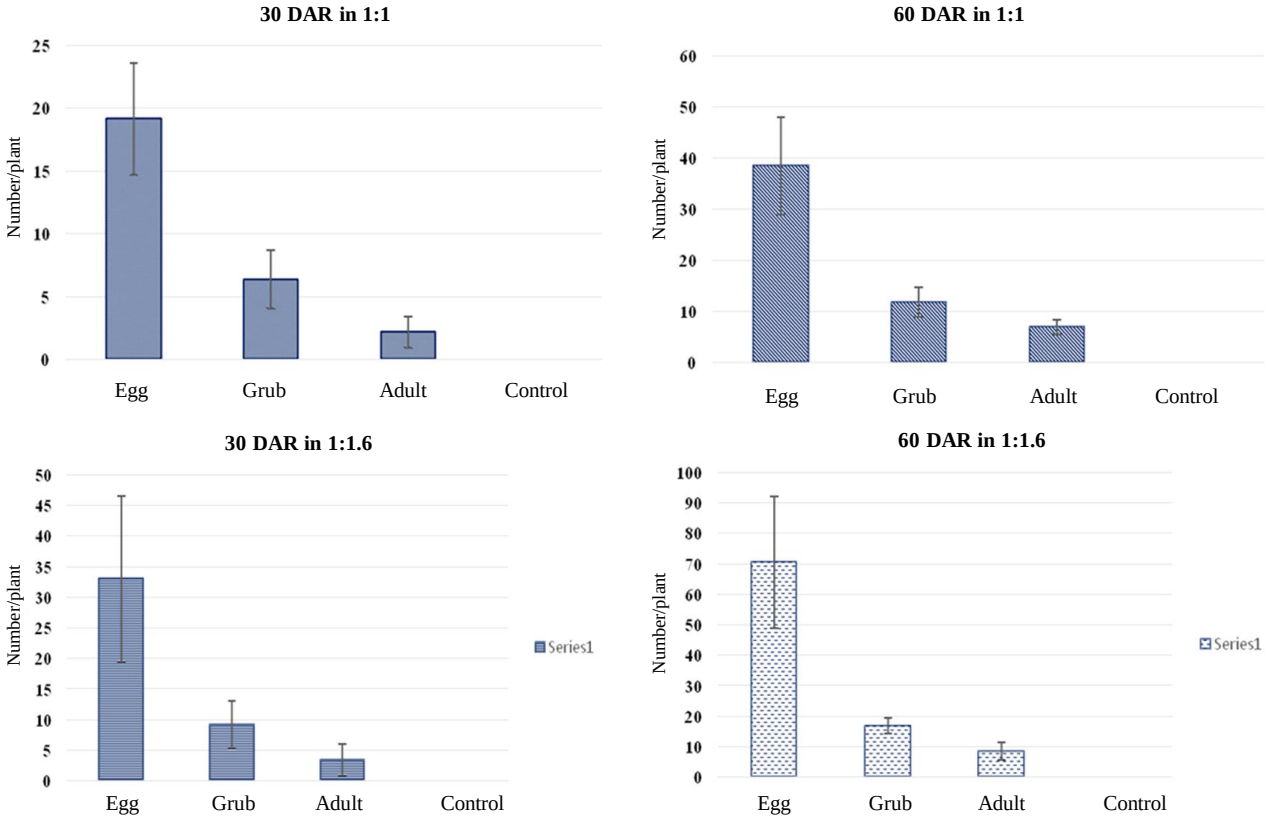


Figure 5. Release effect of *Z. bicolorata* in different male-female ratio on subsequent population increase of eggs, grubs and adults at 30 and 60 days after release

eggs/plant, 16.7 grubs/plant, 30.35 adults/plant and 46.58 male ratio over female while 53.32 female ratio over male at 60 DAR (**Figure 5**).

Among three years, all samples deviated significantly from 50:50 sex ratio ($p<0.005$), while within the same samples, a few were not deviated significantly from the 50:50 sex ratio. However pooled data from within the sample showed slight deviation from the 50:50 sex ratio (chi-square $\div^2$ = 7.9, 13.8, 16.7 (for 2013, 2014, 2015 respectively); df = 1; $p=0.01$). Thus, sex ratio was towards female dominace in each year. The sex ratio in *Z. bicolorata* inclined significantly towards female was reported in previous studies too (Siddhapara 2011, Omkar *et al*. 2013, Omkar *et al*. 2013, Pawar *et al*. 2015). *Z. bicolorata* population increases gradually from June to September and decreases from October onwards and reaches almost negligible in December to January, while mild population occurs during February to March (Dhiman and Bhargawa 2005, Sushilkumar 2005, 2009). Sufficient population build-up during the rainy season is responsible for minimizing Parthenium density and survival of bioagent for future establishment. Female's dominance in sex ratio in *Z. bicolorata* lead good population build-up responsible for subsequent control of Parthenium in future. Bhoopathi *et al*. (2011) considered sex ratio as an important biological parameter to determine the stability of population of *Z. bicolorata* and adaptability of various stages to prevailing biotic and abiotic factors in the field conditions. Less proportion of male lead greater numbers of female emergences in future. Similarly, Visalakshy and Jayanth (2008) reported a female biased sex ratio from a field collected population of *Z. bicolorata.* Hopwood *et al*. (2016) opined natural selection due to ecological differences between the sexes, an alternative to sexual selection as a cause of sexual dimorphism. In our experiments, there was clear effect of Parthenium suppression in the plots where female dominated releases at 1:1.60 were made compared to equal number of male and female releases. Therefore, female dominated population at 1:1.60 male: female sex ratio or even more female dominated sex ratio releases should be opted under biological control programme of Parthenium.

Anderson and Simmons (2006) explained large size in sexual dimorphism a favorable factor in females to produce more eggs while advantageous to males in mating due to smaller size. Larger males and females are preferred as mates over smaller ones while large-sized females produce more progeny

(Omkar and Uzma Afaq 2011). Omkar *et al.* (2013) found that pairs with larger size had higher fecundity, while the egg viability was influenced by the male size only. The offspring of stronger parents are fast developed and have higher survival rate than smaller parents. It is possible due to better nutrient supply by the female (Claessen *et al.* 2003).

Dhileepan *et al.* (2000) evaluated the impact of defoliation using a visual scoring (0 to 100%) by *Z. bicolorata* on *P. hysterophorus* and found 91–100% defoliation resulting in reductions in weed density by 32–93%, plant height by 18–65%, plant biomass by 55–89%, flower production by 75–100%, soil seed-bank by 13–86% and seedling emergence in the following season by 73–90%, however they did not quantified the effect on these parameters based on the release of total number of beetles or release of numbers of male and female adults in different sex ratio. Bhumannavar and Balasubramanian (1998) found that third instar grubs and egg-laying females ingested maximum food. Omkar and Afaq (2011) reported that in adults, females had higher dry food consumption than males but no difference in tissue growth between males and females. They opined that this could be due to diversion of the increased uptake of nutrients and energy resources for ovarian development and egg production in females.

Conclusion

Sex-ratio of bioagent *Zygogramma bicolorata* was found significantly dominated by females along with larger size and more body weight. The female dominated population releases were found helpful to reduce the height, density, biomass and flowers of Parthenium when released at 1:1.60 male: female sex ratio. Therefore, release of female dominated population at 1:1.60 male: female sex ratio or even more were recommended for better establishment of the bioagent and subsequent control of Parthenium.

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