



Biocontrol efficiency of *Zygogramma bicolorata* at different growth stages of *Parthenium hysterophorus*

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Parthenium hysterophorus L. is a highly invasive plant of global significance. It is a herb of neotropical origin which now has spread to many parts of the world (Adkins and Shabbir 2014). 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 hectares of land (Sushilkumar and Varshney 2009, Sushilkumar 2014). It is notorious for causing allergic reactions (Kologi *et al.* 1997) besides a threat to biodiversity and loss of crop productivity (Adkins and Shabbir 2014, Sushilkumar, 2014). In Pakistan, this weed has been spreading very fast since last couple of decades and it has become a major weed of wastelands, fallow lands, roadsides and protected areas where it is rapidly replacing the native flora (Shabbir *et al.* 2012, Mujahid 2015). It is also reported from some cereal and vegetable crops (Safdar *et al.* 2015, Javaid and Anjum 2005) and ornamental plant nurseries (Shabbir *et al.* 2012).

Different control approaches have been used for the management of *Parthenium*. 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 (Krishnamurthy *et al.* 1977). Biological control of *Parthenium* weed is considered to be the most cost effective, environmentally safe and ecologically viable method (Dhileepan *et al.* 2000). It was documented to control *Parthenium* worth of Rs 10 million in terms of herbicide cost after initial release of bioagent *Zygogramma bicolorata* Pallister at Jabalpur, India (Sushilkumar 2006) and it was estimated that this bioagent has checked the spread of *Parthenium* in about eight million hectares of land since its release in India (Sushilkumar and Yaduraju, 2015). In Pakistan, *Z. bicolorata* fortuitously arrived from India to Pakistan, by either flying or eggs or beetles carried on vehicles like it has been suspected

in Nepal (Shrestha *et al.* 2015). Its possible entry in Pakistan was surmised by Sushilkumar (2005) due to its presence in abundance near Wagah border of India and Pakistan (Sushilkumar 2014). This beetle was first reported from the Changa Manga plantation near the district of Lahore (Javaid and Shabbir 2007) and more recently in six more districts including the capital Islamabad (Shabbir *et al.* 2012). *Zygogramma bicolorata* was first introduced to India in 1984 where it became abundant within 3 years after its release, resulting in a significant reduction in *Parthenium* weed densities in localized areas (Jayanth 1987, Jayanth and Bali 1994, Jayanth and Ganga-Visalakshy 1996).

It has been observed in Pakistan that *Parthenium* weed emerges earlier and present well in advance stages of its growth before *Z. bicolorata* appears in mid spring (March). Further, there is no information available on the response of the weed to feeding caused by *Z. bicolorata* in Pakistan. Hence, in this study we assessed the effectiveness of *Z. bicolorata* as a biological control agent, with an emphasis on the response of the weed to the different defoliation pressures and at different growth stages of *Parthenium* weed grown under glasshouse conditions.

Parthenium were sown at a depth of about 2 cm in the fertilized soil contained in porous rectangular trays (50 × 23 × 6 cm l/w/h). After germination, 2-weeks old seedlings of *Parthenium* weed were transplanted from the trays into three sets of earthen pots each containing 6 kg of the fertilized garden soil. One seedling of *Parthenium* plant/pot was transplanted carefully in the middle of pots. First set was labelled as “flowering” because the aim was to apply the biological control agent, *Z. bicolorata* at a time when these plants would be at the flowering stage of their growth. There were four treatments, 1, 2 and 3 pairs of *Z. bicolorata* adults to be applied on replicated *Parthenium* plants and fourth one was kept

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as a control. A total of twelve pots with 3 replicates for each treatment were distributed randomly on the concrete platforms inside the glass house.

Second set was labelled as ‘pre-flowering’ and third set was designated as ‘young plants’. Same four treatments were made as described above. After six weeks of initial transplantation, plant batches in three different growth stages, viz. flowering, pre-flowering and young were available for further experimentation.

In order to apply *Z. bicolorata*, several field visits were made around the wastelands of Lahore for the collection of adult beetles. Once collected, the adult beetles were applied in three different pairs (1-pair, 2-pairs and 3-pairs) and at three different growth stages (Flowering, pre-flowering and young) of *Parthenium* weed at a same time in the first week of May 2015. To stop the escape of adult beetles, the individual pots were covered with an insect proof net cage. Three replicates at each growth stage (flowering, pre-flowering and young) were kept as control where no beetle was applied but plants were caged.

Throughout the experiment, the level of insect damage inflicted upon the *Parthenium* weed plants was monitored on a weekly basis. A scale was developed to assess the degree of defoliation damage (%) caused by the beetle and its larvae. This was based on the number of leaves per plant damaged at the various growth stages (i.e. if two leaves were found to be eaten at the six-leaf stage, a 33% damage level was designated, similarly 50 and 66% level designated if three or four leaves being damaged, respectively at six leaf stage. After 12-weeks of agent application, harvesting of the *Parthenium* weed plants was done manually by uprooting the complete plant carefully along with roots. After that plant height, shoot length, root length, number of flowers was recorded. After this individual treatment plants were packed in brown paper bags and placed in an oven set at 60°C for dry biomass determination.

Impact on *Parthenium*

The defoliation level of *Parthenium* increased gradually with the time and this effect was seen in all treatment pairs of the agent applied. The damage (defoliation %) recorded ranged 44-57% at flowering, 40-54% at pre-flowering and 100% at young stage of the weed (Fig. 1). At the end of experiment, on average the biological control agent in different pairs inflicted a defoliation of 69.3, 68.3 and 97.6% when applied at the flowering, pre-flowering and young stages, respectively (Fig. 1).

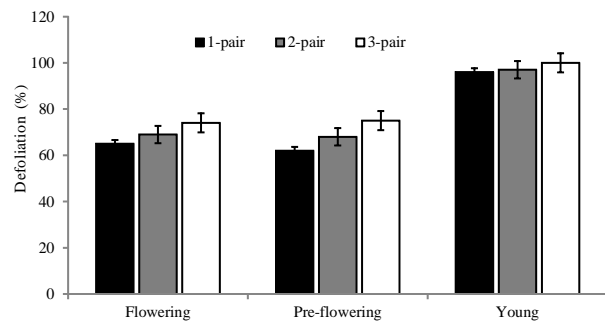


Fig. 1. Graphical representations of damage caused by the different pairs (1, 2 and 3) of the biological control agent, *Z. bicolorata* when applied at different growth stages (flowering, pre-flowering and young) of *Parthenium* weed

The number seeds produced per plant gradually decreased as the density (number of pairs) of *Z. bicolorata* adults increased. The number of seeds produced by *Parthenium* weed was reduced to 60.8, 73.9 and 81.6% when *Z. bicolorata* was applied at flowering stage in ratios of 1, 2 and 3 pairs, respectively (Fig. 2). No seed was produced even when *Z. bicolorata* applied in the lowest ratio (1-pair/plant) at the young stage of weed growth (Fig. 2). In *Lythrum salicaria* (L.) defoliation due to Chrysomelid beetles resulted in significant reduction in seed production by more than 99% (Blossey 1992).

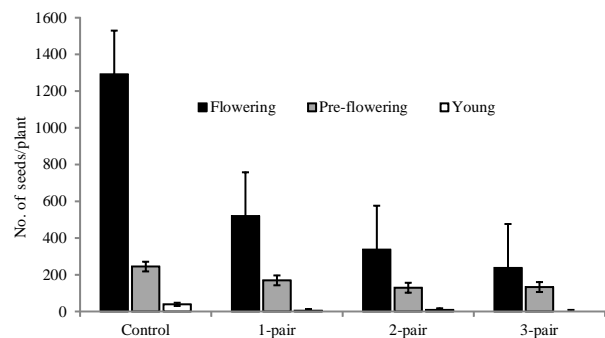


Fig. 2. Effect of different pairs (1, 2 and 3) of the biological control agent, *Z. bicolorata* on total number seeds/plant produced by *Parthenium* weed when applied at different growth stages (flowering, pre-flowering and young)

All three different pairs (1, 2 and 3) of *Z. bicolorata* exhibited suppressive effects on the shoot length of the *Parthenium* weed (Fig. 3). When *Z. bicolorata* was applied in 3-pairs at the flowering, pre-flowering and young stages, the shoot length was decreased to 37, 19.4 and 43%, respectively as compared to the control plants. The above ground dry biomass of *Parthenium* weed plants grown at the different growth stages was significantly and

progressively reduced with increasing density of the biological control agent, *Z. bicolorata*. This gradual trend in biomass reduction was similar at flowering and pre-flowering growth stages of *Parthenium* weed as compared to control. The dry biomass of *Parthenium* weed was reduced to 41.3, 62 and 70.6% when *Z. bicolorata* was applied at flowering stage in ratios of 1, 2 and 3 pairs, respectively (Fig. 3). However, at the young stage ca. 90% reduction in biomass was recorded in all three densities of the *Z. bicolorata* applied (Fig. 3). The effectiveness of the agent was found to be density dependent, as defoliation and seed suppression was highest when three pairs of *Z. bicolorata* were applied at different growth stages of the weed. In field this will depend upon the population dynamics of agent and suitable climatic conditions as exhibited by *Z. saturalis* on rag weed (*Ambrosia artemisiifolia* L.) a close relative of *Parthenium* weed (Reznik *et al.* 1994).

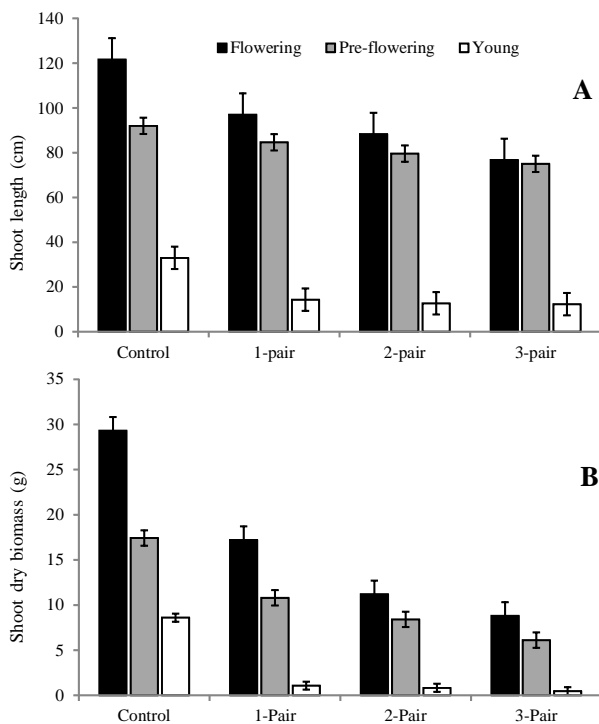


Fig. 3. Effect of different pairs (1, 2 and 3) of the biological control agent, *Z. bicolorata* on (a) shoot length and (b) shoot dry biomass of *Parthenium* weed when applied at different growth stages (flowering, pre-flowering and young).

The defoliation caused by *Z. bicolorata* had an immediate negative effect upon the weed performance and as a consequence the growth, reproduction and fitness of the *Parthenium* weed can be negatively affected. Prins and Verkaar (1992) also found similar trends in their study on *Z. bicolorata*. In the current study, *Z. bicolorata* caused 90-100%

defoliation resulting in significant reduction in weed height, biomass and seed production. In central Queensland, Australia, some similar results were also documented by Dhileepan *et al.* (2000). Likewise, feeding by an introduced *Z. saturalis* reduced the biomass and plant height in ragweed (Kovalev and Medvedev 1983).

Zygogramma bicolorata is an effective biocontrol agent that can significantly reduce the vegetative and reproductive growth of *Parthenium* weed. However, the effectiveness of the biological control *Z. bicolorata* can be further enhanced if it is applied at the early growth stages (young or pre-flowering) of *Parthenium* weed.

SUMMARY

Parthenium hysterophorus L. (Asteraceae) commonly known as *Parthenium* weed, is a highly invasive weed that is considered as environmental, medical, and agricultural hazard. The objectives of this study was to test the impact of *Zygogramma bicolorata* Pallister (Coleoptera: Chrysomelidae), a classical biocontrol agent, in different ratios (1-pair, 2-pairs and 3-pairs) at three different growth stages (flowering, pre-flowering and young) of *Parthenium* weed to estimate the effectiveness of this agent in reducing its growth and reproduction potential. The damage (defoliation %) recorded was between 44-57% at flowering, 40-54% at pre-flowering and 100% at young stage of the weed. The defoliation significantly reduced the weed biomass, height and seed production. The damage inflicted by *Z. bicolorata* was more pronounced when it was applied in higher density and at early growth stages of the weed.

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