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Impact of *Zygogramma bicolorata* on vegetative and reproductive performance of *Parthenium hysterophorus* in Nepal

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ABSTRACT

Parthenium hysterophorus Linn. is one of the most aggressive, invasive weeds threatening natural and agricultural ecosystems in Nepal. Leaf feeding beetle, Zygogramma bicolorata Pallister (Coleoptera: Chrysomelidae), is regarded as a potential candidate for biological control of Parthenium weed. Considering the fact effectiveness of Z. bicolorata against P. hysterophorus was evaluted in Nepal. Z. bicolorata caused 98.25 % defoliation of P. hysterophorus reducing 38.88 % plant height, 27.29 % plant width, 26.25 % root length, 12.33 % leaves, 40.58 % shoot biomass and 36.59 % root biomass in the period of 90 days. The flower production and soil seed bank were reduced by 50.22 % and 40.29 %, respectively. Z. bicolorata was an efficient bio-control agent with a significant negative effect on the vegetative and reproductive performance of the noxious weed P. hysterophorus.

Key Words*: Z. bicolorata, P. hysterophorus, Defoliation, Plant height, Root biomass and Seed bank.*

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I. Introduction

Parthenium hysterophorus Linn. (Asteraceae; Heliantheae), generally known as parthenium, is an herbaceous weed plant with short life cycle. *P. hysterophorus* is a pernicious weed with a huge negative impact on agricultural and natural ecosystems, including human health and it is characterized by its invasiveness with high spreading potentiality (Adkins and Shabbir, 2014; Shrestha et al., 2019). The weed is highly prolific, with profuse and continuous flowering till senescence producing 15,000 to 100,000 seeds per plant (Gnanavel, 2013). The weed is native to tropical America and is now problematic in tropical and subtropical regions. The weed has been reported from 96 countries worldwide including Africa, Asia, Europe, North America, Oceania and South America (CABI, 2020a). *P. hysterophorus* was reported for the first time in Nepal from Trishuli Valley in 1967 (Tiwari et al.,

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2005) and it was suspected that weed came to Nepal from India with contaminated seeds and vehicles (Mishra, 1991). Parthenium has been reported as dominant weed species from various regions of Nepal, even recorded from an altitude of 2,000 m asl (Shrestha et al., 2019).

Various cultural, mechanical, chemical and biological management approaches are followed for control of noxious parthenium weed. Biological control of the *P. hysterophorus* with herbivorous insect pests, including *Z. bicolorata* is one of the most popular, sustainable and environment friendly management options (Kumar, 2009). In Australia, sustainable management of parthenium with biological control started in 1977 and nine host-specific insect species along with *Z. bicolorata* were imported from Mexico during 1980 (McFadyen and McClay, 1981). Similarly, *Z. bicolorata* was introduced in India along with *S. lutulentus* Dietz (Coleoptera: Curculionidae) and *E. strenuana* (Walker) (Lepidoptera: Tortricidae) for biological control of parthenium weed (Dhileepan and Strathie, 2009; Kumar, 2009). There is no record that *Z. bicolorata* had been purposefully introduced in Nepal for management of *P. hysterophorus*, but this insect has been recorded from different parts of the country, probably fortuitously came from India (Shrestha et al., 2010; Shrestha et al., 2011). The insect has been spread into south Asia, covering India, Pakistan, Sri Lanka, Bangladesh and Nepal. CLIMEX model based on the current distribution suggests that the geographic range of this agent can extend in India and Pakistan and all of Bangladesh and Sri Lanka, and parts of Nepal are climatically suitable for *Z. bicolorata* (Dhileepan and Senarate, 2009).

The beetle, *Z. bicolorata,* is commonly known as the Mexican beetle, but also called as parthenium beetle (CABI, 2020b). Larval and adult feeding on parthenium results in skeletonization, defoliation and reduction in flowers and seed production. *Z bicolorata* can cause 100% defoliation of parthenium, resulting in reduced weed density, plant height, plant biomass, flower production and soil seed bank (Dhileepan et al. 2000a). The efficacy of this insect on *P. hysterophorus* had been studied in various countries, including Australia, India and Pakistan. Effects of defoliation by *Z. bicolorata* on parthenium weed were studied in field cages by Dhileepan et al. (2000b) in Australia. They found that feeding of *Z. bicolorata* caused damage to meristems of parthenium, resulting in shorter plant height and altered branching pattern. *Z. bicolorata* caused 92 % defoliation in 90 days and reduced plant height by 27 %, root length by 56 %, root biomass by 69 %, shoot biomass by 81 % and flower production by 83 % in field cage conditions. In India, *Z. bicolorata* caused 85–100 % defoliation of parthenium plant and reduced up to 99.5 % weed density in natural conditions in Bangalore region (Jayanth and Bali, 1994; Jayanth and Visalakshy, 1996). Similarly, in Pakistan impact of *Z. bicolorata*, was studied on *P. hysterophorus* and reported defoliation significantly reduced the weed biomass, plant height and seed production of parthenium (Shabbir et al., 2016). Such efficacy evaluation of *Z. bicolorata* on *P. hysterophorus* needs to be conducted before utilizing it as biocontrol agent of the weed within Nepal. Considering this fact, the present study was undertaken to understand the effects of *Z. bicolorata* on vegetative and reproductive performance of *P. hysterophorus* inside the screen houses of the National Entomology Research Center of Nepal Agricultural Research Council at Lalitpur, Nepal.

II. Materials and Methods

Study site

The effectiveness of *Z. bicolorata* as a biological control agent against *P. hysterophorus* (Picture 01) was evaluted at National Entomology Research Center under Nepal Agricultural Research Council, Khumaltar, Lalitpur, Nepal during May - September, 2018. The study location's GPS coordinate are 27°39'19.0"N 85°19'33.8"E and the altitude is 1308 m asl.

Study organisms

Seeds of *P. hysterophorus* (Picture 02A) were collected from matured plants. The seeds were collected from plants grown on wastelands inside Nepal Agricultural Research Council, Khumaltar, Lalitpur, Nepal (27°39'19.9"N 85°19'38.4"E) during November, 2017. The seeds were dried, cleaned and stored in air-tight plastic bags. The seeds were kept at 4°C temperature in the refrigerator until used. The seeds were sown in plastic trays of 30 cm X 20 cm X 6 cm of length, breadth and depth during the first week of May, 2018. The soil was sterilized at 80 °C for 30 minutes in hot air oven. The trays were kept inside the screen house and regular irrigation was provided with water can. A two-week-old seedling of *P. hysterophorus* was used in the experiment.

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A B

Picture 01. Experiment within screen houses (A) and *Z. bicolorata* defoliated *P. hysterophorus* (B)

The initial culture of *Z. bicolorata* was collected from Chitwan district of Nepal (N 027°34.110', E 084°43.792'). The insect was reared at laboratory conditions in rearing cages made of transparent plastic boxes of 22.7 cm X 16.3 cm X 9.0 cm length, breadth and height. Parthenium plants were grown in screen houses for food of the insect. The petiole of fresh parthenium leaves were wrapped with moistened cotton in order to prevent early senescence. Bunch of such parthenium leaves was provided in rearing cages for adults and larvae of *Z. bicolorata.* Provisions of ventilation inside the rearing boxes were made with 10 cm X 5 cm window on lid. The window was covered with black muslin cloth to prevent escape of the insect. Old leaves of parthenium in the rearing boxes were removed and fresh food was provided daily. The eggs laid by adult insects were collected and used for rearing of the insect. The eggs were kept for hatching in 9 cm petri plate. The first instar larvae of *Z. bicolorata* were moved to rearing cages with the help of fine camel hairbrush. When larvae fully grown and stopped feeding collected and kept for pupation in soil in plastic boxes. The soil was sterilized in hot air oven at 80°C for 30 minutes before using as pupation media. The necessary moisture in soil was provided with distilled water. The adult emerged from pupa were collected and used for continuous multiplication of the insect.

Experimental setup

The efficacy of *Z. bicolorata* against *P. hysterophorus* was studied inside the screen houses of dimensions 2.5 m X 2.5 m (Picture 01A). Four screen houses were utilized; two for evaluation of *Z. bicolorata* and two were kept as control without *Z. bicolorata*. The soil inside screen houses was free from previous infestation of *P. hysterophours*. The land was prepared ploughing and 20 kg of well decomposed compost manure was added as a source of nutrients for *P. hysterophorus* plants. Two week old seedlings of *P. hysterophorus* grown on plastic trays were transplanted in the screen houses at the spacing of 50 cm X 50 cm. A total of 25 *P. hysterophorus* plants were maintained inside each screen house throughout the experimental period. Plants were regularly irrigated twice a week. One day old adults of *Z. bicolorata* were used for evaluation; 100 beetles were released inside a single cage. The insect was released in two cages while two cages were kept free from *Z. bicolorata* infestaion throughout the experimental period.

Observation

Observation on the plant height, plant width and percent leaf defoliation was recorded at 30, 60 and 90 days after the insect release. Observation was recorded from 10 randomly sampled plants in each insect released as well as control cages. The plant height was measured as the highest point from the ground level. The plant width was measured perpendicular to plant height at the widest part of the canopy. Percent leaf defoliation was recorded based on the total number of leaves and number of insect-damaged leaves per plant at the time of observations.

Similarly, 90 days after release of the insect, observation on the number of leaves, number of flowers, root length, shoot and root biomass was recorded. The ten random plants were pulled out carefully and kept in separate plastic bags. These plants were brought to laboratory for observations. The number of leaves and flowers produced per plant were counted visually. The root length was measured and separated from shoot with the help of the secateur. Each plant along with its root was

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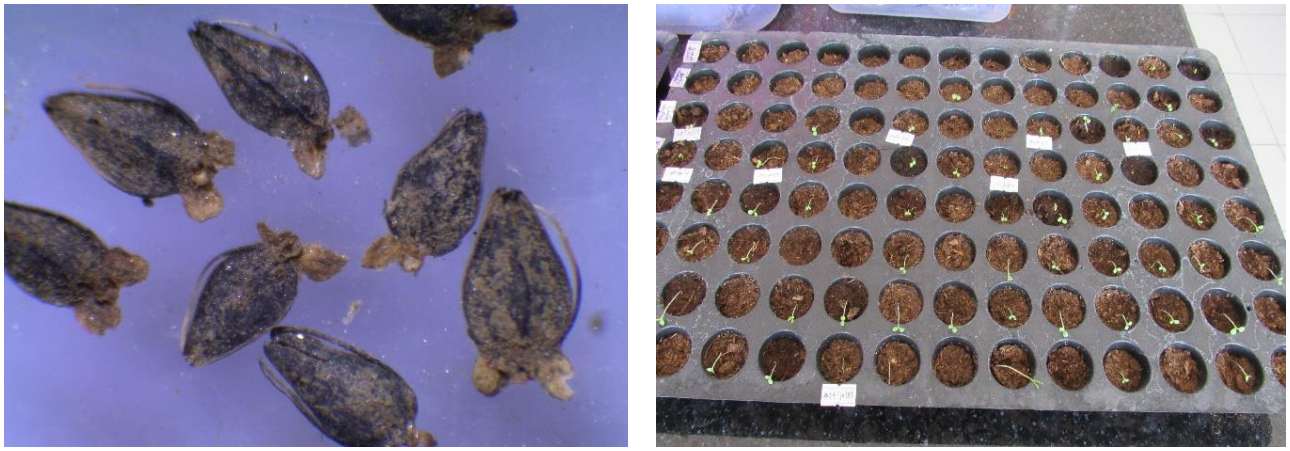
placed in a separate tray and dried in a hot air oven at 60°C for 48 hours until further weight was not changed. Weight of above ground parts was recorded as shoot biomass while, underground part was root biomass.

Measurement of soil seed bank

Soil seed bank inside the cages (experimental site) was determined during October - November. Eight random soil samples (10 cm diameter, 5 cm depth) were collected for control and *Z. bicolorata* released cages. These samples were dried in plastic trays of dimension 30 cm X 20 cm X 6 cm at room temperature. The soil samples were sieved through mess of 2 mm size to remove plant roots, large pebbles, and other unwanted materials. Soil seed extraction method described by Gonzalez and Ghermandi (2012) was followed to extract *P. hysterophorus* seeds from soil samples. NaCl solution was prepared in 2000 ml capacity glass beakers by dissolving 350 g of NaCl in 1000 ml distilled water. Each soil sample dissolved in separate beakers containing NaCl solution by stirring with a glass rod for 5 minutes and settling for 30 minutes. The supernant was filtered through whatman no 1 filter paper The seeds collected on the filter paper were dried in a hot air oven at 35°C for 24 hours. The parthenium seeds were identified, separated and counted under stereo-microscope (Best scope, BS-3040T) by their small size, triangular shape and blackish appearance (Picture 02A). The number of seeds/m^2 was estimated using the formula: Number of seeds/m^2 = (Number of seeds/core) x (10000/Area of core in cm^2). The counted seeds were kept in sealed plastic bags and stored at 4°C in the refrigerator before germination tests.



A B

Picture 02. Soil extracted *P. hysterophorus* seeds (A) and germination test on nursery tray (B)

The seeds' viability was determined by performing germination test on the plastic nursery trays (Picture 02 B) with cocopit as growing medium at laboratory conditions with temperature of 26±2ºC and relative humidity of 70±10%. The capacity of each cavity on the nursery tray was 20 ml. The seeds were washed with one percent sodium hypochlorite solution and followed with distilled water. One hundred seeds from each sample were shown individually on each cavity of nursery tray. Observations on germination of the seeds were recorded daily and continued for one week. The percent germination of *P. hysterophorus* seeds was calculated.

Data anlysis

All the observations recorded were entered in a Microsoft excel sheet. The two-sample t-test was performed to compare the plant parameters between *Z. bicolorata* released and control in Genstat Discovery Edition 4 and presented as the result section.

III. Results and Discussion

The defoliation level of *P. hysterophorus* was found to increase gradually with an increase in period for feeding by adults and larvae of *Z. bicolorata* (Figure 01). The percent leaf defoliation was recorded 39.15 % at 30 days after release of the insect. The defoliation level reached 91.75 % after 60 days from the release of the insect. The leaves which were found partially damaged at 30 days were found heavily skeletonized after 60 days. At the end of the experiment 98.25 % of leaves were found

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defoliated and all the leaves were found completely skeletonized. The defoliation of *P. hysterophorus* in control plots were not observed throughout the experimental period.

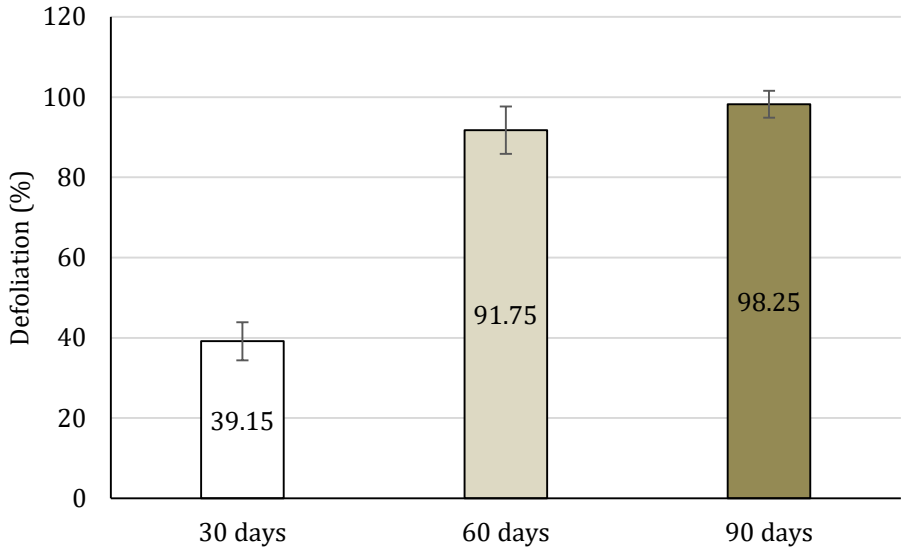

Figure 01. Percent leaf defoliation caused by *Z. bicolorata* on *P. hysterophorus* (mean ± SD) at 30, 60 and 90 days after the insect release.

We found the adults *Z. bicolorata* initiated to defoliate *P. hysterophorus* leaves immediately after release. The defoliation level that remained below 40 % up to 30 days increased to more than 90 % after 60 days and reached 98 % by 90 days. The increase in defoliation of the *P. hysterophorus* after 30 days could be accounted for an increase in larval population of *Z. bicolorata* and continuous feeding for a longer period by both adults and larvae. Shabbir et al. (2016) reported similar finding that defoliation of *P. hysterophorus* leaves reached up to 100 % in 12 weeks period. Hasan et al. (2020) reported that complete defoliation of *P. hysterophorus* was achieved in 80 days when adults were released. They also noted a significantly higher number of eggs, larvae, and adults, 20 days after *Z. bicolorata* release, resulting in earlier population build-up when the adults of *Z. bicolorata* was released compared to larval release. We found 134.36 % increase in percent defoliation at 60 days compared to 30 days after insect release. Kanagwa et al. (2020) reported leaves eaten by *Z. bicolorata* after 42 days increased by 75-107 % compared to damage level at 28 days after release of the insect.

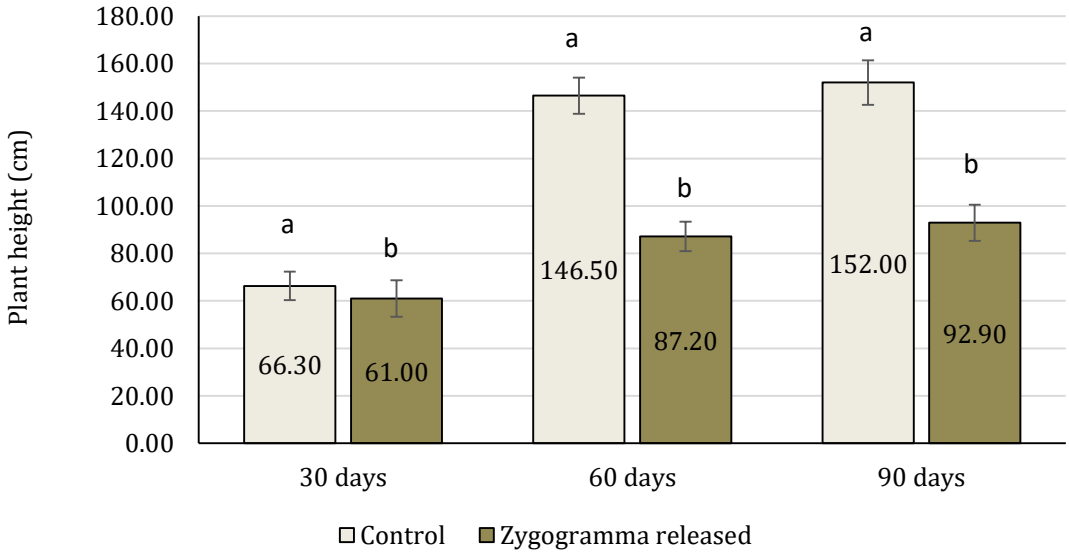

Figure 02. Plant height of *P. hysterophorus* (mean ± SD) at 30, 60 and 90 days after releasing *Z. bicolorata* adults. *t*-test: for each duration, means with the same letter are not significantly different from each other (p>0.05).

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The plant height of *P. hystrophorus* was found significantly reduced under *Z. bicolorata* infestation compared to control (Figure 02). The average plant height of *P. hysterophorus* was 61.00 cm after 30 days defoliation by *Z. bicolorata* while the control recorded plant height of 66.30 cm. The plant height was found to be reduced by 7.99 % after 30 days. Similarly, the plant height was recorded 87.20 cm after 60 days defoliation by *Z. bicolorata,* while undefoliated plants were 146.50 cm high. The plant height reduction over control was found 40.48 % on 60th day. The plant height growth was slower in control and *Z. bicolorata* infested plots 60 days afterward, recording 92.90 cm and 152.00 cm in infested and control plots, respectively, at 90 days after inoculation of the insect. The height of plant was found to be reduced by 38.88 % at 90 days.

Similarly, the average plant width of *P. hysterophorus* was found significantly reduced by 22.87 % and 27.90 % in *Z. bicolorata* infested plants compared to control plants during 60 and 90 days observations, respectively (Figure 03). The plant width was found not significantly differed in 30 days after inoculation of the insect. The plant width was 116.30 cm in control plots and 89.70 cm in *Z. bicolorata* released plots at 60 days after inoculation of the insect. Average plant width was recorded 117.20 cm and 84.50 cm, respectively, in control and the insect released screen houses after 90 days of infestation.

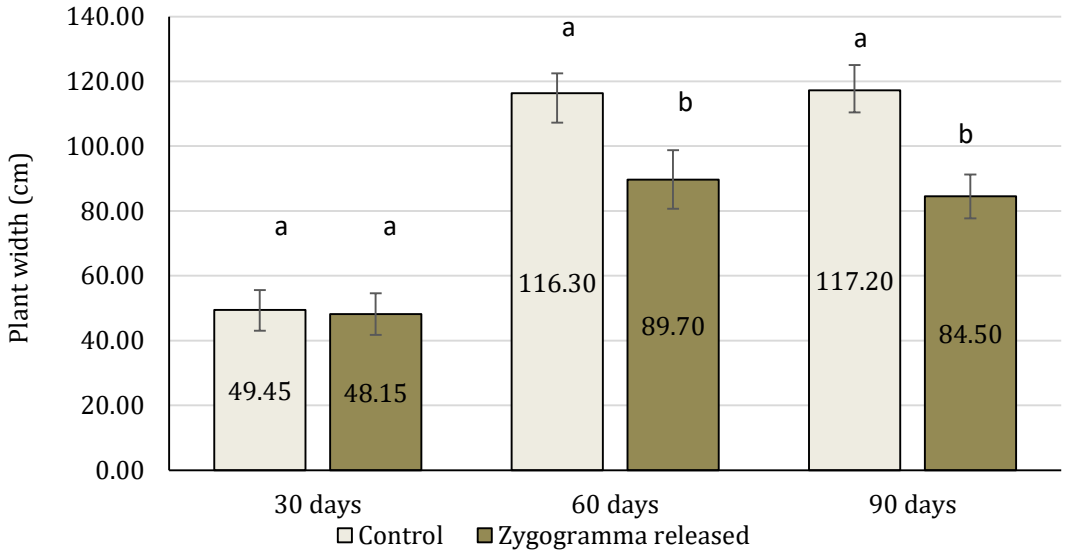

Figure 03. Plant width of *P. hysterophorus* (mean ± SD) at 30, 60 and 90 days after releasing *Z. bicolorata* adults. *t*-test: for each duration, means with same letter are not significantly different from each other (p>0.05).

Z. bicolorata showed a suppressive effect on *P. hysterophorus* and growth was slower with reduced plant height and width. Dhileepan et al. (2000a) reported reduced primary stem height altered branching pattern of *P hysterophorus* due to damage of meristem caused by continuous feeding on stem tips by *Z. bicolorata*. They found that sustained defoliation for 90 days reduced plant height by 27 %, slightly less than present findings of 38.88 %. The reduction in plant height due to feeding of *Z. biclorata* was also reported by Hasan et al. (2020) and Kanagwa et al. (2020). We found plant height and width did not differ much till 30 days after release of the insect. Dhileepan et al. (2000a) reported lack of negative effect on *P. hysterophorus* in the earlier stage of development when herbivory pressure by *Z. bicolorata* was low. Parthenium could compensate for shorter duration defoliation due to higher photosynthetic rate of emerging leaves (Cox and McEvoy, 1997).

The impact of sustained defoliation of *Z. bicolorata* for 90 days was found significantly evident on production of leaves (Figure 04) and flowers (Figure.05). The average number of leaves per plant was reduced by 12.33 %, recording 41.25 leaves per plant in treated plots, whereas control recorded 47.05 leaves per plant. The number of flowers produced on control plants were as high as 6649 flowers/plant whereas, the flower production was reduced to 3310 flowers/plant in *Z. bicolorata* releases plants. The 50.22 % of flower production was reduced due to defoliation by *Z. bicolorata* for 90 days.

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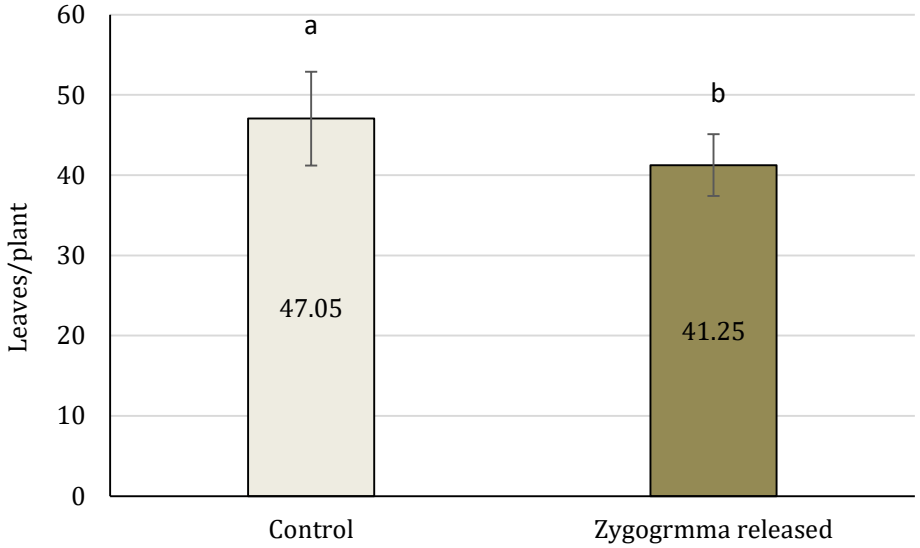

Figure 04. Number of leaves (mean ± SD) per plant after 90 days of Z. *bicolorata* release. *t*-test: means with the same letter are not significantly different from each other (p>0.05).

The average number of leaves and flower production was decreased by the impact of *Z. bicolorata* feeding in the present study. Reduction in leaf and flower production was also reported by Dhileepan et al. (2000a), Hasan et al. (2020) and Kanagwa et al. (2020) due to *Z. bicolorata* feeding. The alteration in branching pattern and damage in meristem could cause reduction in leaf production in *P. hysterophorus* (Dhileepan et al., 2000b). The reduction in flower production due to *Z. bicolorata* is associated with feeding behavior the insect, which tends to congregate and feed on tender leaves, terminal and axillary buds, leading to stunted growth and fewer flower production (Manjunath, 2010; McConnachie, 2015). The flower production reduction in defoliated plants could also due to re-allocation of resources to reestablish root: and shoot ratio in plant (Dhileepan et al. 2000b).

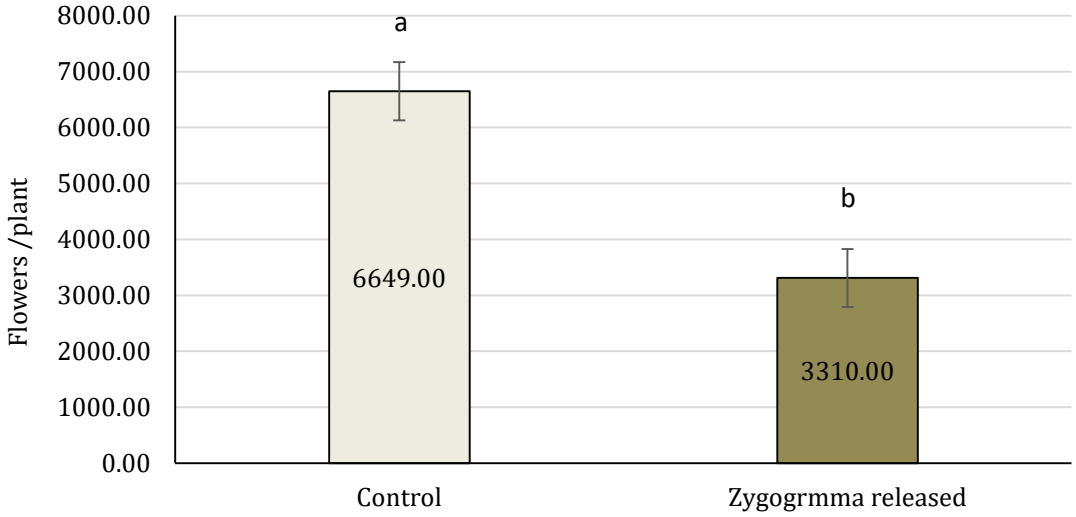

Figure 05. Number of flowers (mean ± SD) per plant after 90 days of Z. *bicolorata* release. *t*-test: means with same letter are not significantly different from each other (p>0.05).

The dry biomass of *P. hysterophorus* was found to be reduced significantly including both shoot and root biomass when *Z. bicolorata* defoliated for 90 days (Figure 06). The shoot biomass of above ground part was found to be reduced by 40.58 % while; root biomass (underground part) was found to be decreased by 36.59 %. The mean shoot and root biomass per plant in *Z. bicolorata* infested plants were recorded, 40.70 g and 5.20 g, respectively. The plants in control plots recorded average shoot and root biomass as 68.50 g and 8.20 g per plant, respectively. The average root length of *P. hysterophorus* was found 20 cm long in control plants whereas, it was 14.75 cm long in *Z. bicolorata* infested plants (Figure 07). The root length was found to decrease by 26.25 % due to leaf feeding of *Z. bicolorata*.

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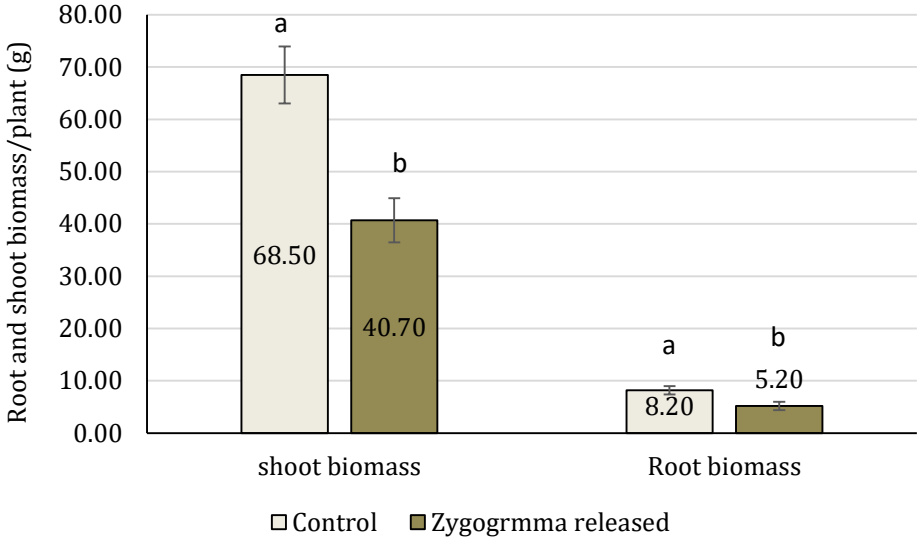

Figure 06. Impact of *Z. bicolorta* on shoot and root biomass of *P. hysterophorus* 90 days after release of *Z. bicolorata* adults. *t*-test: for each biomass parameter, means with the same letter are not significantly different (p>0.05).

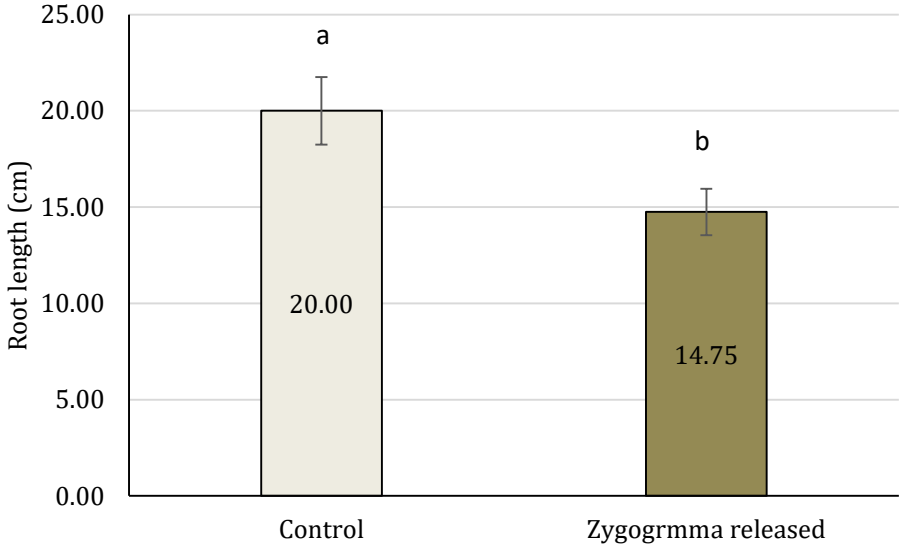

Figure 07. Root length of *P. hysterophorus* (mean ± SD) 90 days after release of Z. *bicolorata* adults. *t*-test: means with same letter are not significantly different from each other (p>0.05).

The reduction in plant biomass (both shoot and root biomass) due to *Z. bicolorata* defoliation was found significant in present study. Such reduction in biomass production was also reported by Dhileepan et al. (2000a), Shabbir et al. (2016), Hasan et al. (2020), and Kanagwa et al. (2020). Dhileeepan et al. (2020b) reported minimum period of eight weeks of continuous defoliation was required to cause a significant reduction in root and shoot biomass. In our present study, the defoliation was sustained for 90 days which was a sufficient period to cause reduction in plant biomass. The less resource allocation to root may cause of slow development of root, ultimately reducing biomass of root in *Z. bicolorata* damaged plants (Arredondo and Johnson, 1998).

Significant difference in soil seed bank was recorded between plots with and without *Z. bicolorata* (Figure. 08 A). The soil samples showed the seed bank of 2282/m^2 in *Z. bicolorata* released plots whereas, the control plots recorded the seed bank of 3822/m^2. This indicated that the soil seed bank was reduced by 40.29 % when *Z. bicolorata* defoliated *P. hysterophorus* for 90 days. The germination of the seed between the plots with and without *Z. bicolorata* was not differed significantly (Figure. 08 B). The germination percent of the seed ranged between 90.83 % to 94.12 %.

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We found the *Z. bicolorata* significantly reduced the soil seed bank of the *P. hysterophorus* by 40.29 %. The soil seed bank was found to reduce due to feeding on flowers by the insect along with reduced plant height and canopy due to defoliation. Dhileepan et al. (2000a) reported in completely defoliated *P. hysterophorus* plants adult oviposited on flower heads and emerged larvae fed on flower thus preventing seed production. *P. hysterophorus*is is a very prolific seed producer, producing up to 25,000 seeds/plant, leading to large seed bank in the soil (Monaco et al., 2001). Seed can remain intact for a long time in soil with around 50 % of the buried seed remaining viable for 6 years (Navie et al., 1998). Thus, defoliation of *P. hysterophorus* need to be continued for several years until the existing soil seed bank is practically depleted.

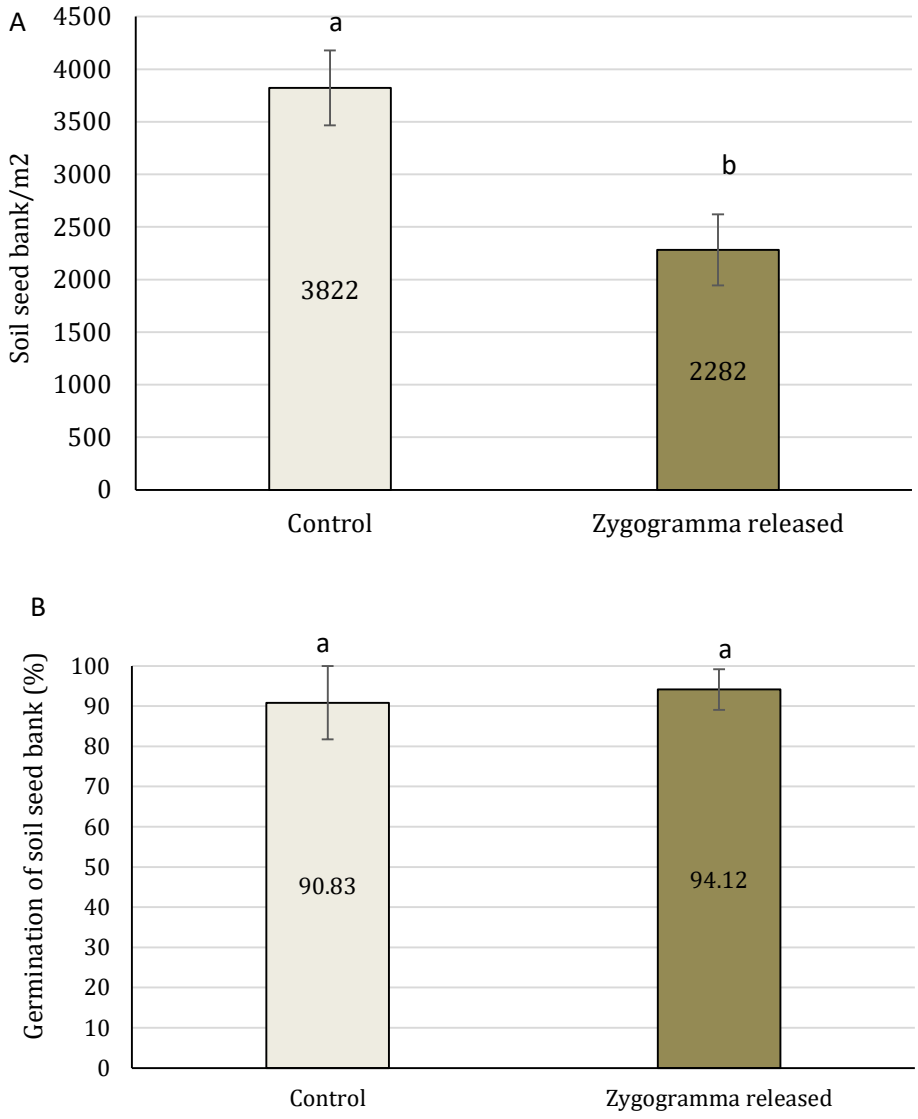

Figure 08. Impact of *Z. bicolorta* on soil seed bank of *P. hysterophorus* (A) and germination percent of soil seed bank (B). *t*-test: for both parameters, means with same letter are not significantly different from each other (p>0.05).

IV. Conclusion

Our present study found that *Z. bicolorata* can completely defoliate the *P. hysterophorus*, adversely affecting plant height, canopy, leaf production, flower production, plant biomass, and ultimately soil seed bank. Thus, *Z. bicolorata* is an efficient bio-control agent of noxious weed *P. hysterophorus,* having significant negative effect on vegetative and reproductive performance of the weed. The insect can be utilized in future management programs of parthenium weed in Nepal as efficient and self-sustainable bio-control agent.

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