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ORIGINAL RESEARCH ARTICLE

Life cycle of *Zygogramma bicolorata* and its effectiveness as biocontrol of *Parthenium hysterophorus* in Lamjung, Nepal

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ABSTRACT

The invasive weed *Parthenium hysterophorus* L. poses a significant ecological and agricultural threat in Nepal, necessitating effective and sustainable management strategies. This study investigates the life cycle and biocontrol efficacy of *Zygogramma bicolorata* Pallister against *P. hysterophorus* under greenhouse conditions in Lamjung, Nepal. Detailed observations on developmental biology revealed an average life span of 62–78 days from egg to adult, with females showing longer longevity (43.3±0.72 days) than males (37.0±0.94 days). Each female laid an average of 650±21.45 eggs during its lifespan, with 84.08% hatching success, 81.32% pupal recovery, and 78.69% adult emergence. The beetle exhibited four distinct larval instars, each showing progressive growth and color changes, followed by pupation at 3–5 cm soil depth. Feeding potential varied with the developmental stage and beetle density. Feeding trials revealed that both adults and larvae caused significant defoliation of Parthenium with higher beetle densities leading to faster defoliation. Defoliation was faster at the seedling stage than the flowering stage, with five beetle pairs completing defoliation in 1.13 days and 6.67 days, respectively, compared to 6.67 and 15.67 days with two pairs. Larvae were particularly voracious feeders, contributing more substantially to defoliation than adults. Based on these findings, *Z. bicolorata* shows strong potential as a biocontrol agent against *P. hysterophorus*, and further field-based research is recommended to evaluate its establishment and effectiveness across Nepal's diverse agro ecological zones.

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INTRODUCTION

Parthenium hysterophorus L. (family: Asteraceae) is a neotropical annual herb with a pantropical distribution and is recognized as a highly invasive exotic weed (Evans, 1997). Commonly known as parthenium, carrot grass, congress grass, star weed, and white top (Shah *et al*., 2013), it grows rapidly, reaching up to 2 m in height (Haseler, 1976), and poses severe threats to crop productivity, biodiversity, and human and animal health (Kanagwa *et al*., 2020). The weed is assumed to be native to the tropical and subtropical Americas, possibly in the Gulf of Mexico, including the southern United States and Caribbean islands, or in northern Argentina and southern Brazil (Dale, 1981). It has successfully invaded Asia, Africa, and Australia due to its strong reproductive ability, persistent seed bank, adaptability to diverse habitats, and allelopathic interference with neighboring plants (Hassan *et al*., 2018). Introduced from India in the 1960s, *P. hysterophorus* has been actively spreading throughout Nepal for the past 25 years or more, primarily in the Terai, Siwalik, and Middle Mountains (Tiwari *et al*., 2005; Shrestha *et al*., 2019). Shrestha *et al*. (2019) found that this weed is more prevalent in fallow-grazing areas, followed by crop grounds. The weed competes with other plants in the invaded areas primarily by producing a variety of allelochemicals from its underground and

aerial parts, which exerts phytotoxic effects (Batish *et al*., 2005; Singh *et al*., 2005; Khaliq *et al*., 2015) by inhibiting seed germination as well as retarding morphological and physiological activities of nearby plants (Tefera, 2002; Pandey, 2009; Hassan *et al*., 2018). Its ability to thrive in a variety of habitats, strong reproductive and regenerative power, persistent seed bank, and allelopathic potential make management problematic (Kohli *et al*., 1998; Dhileepan, 2009) and integrated strategies are required to prevent its epidemic spread. Its rapid proliferation has led to significant ecological impacts and economic losses, making it a major environmental issue across the tropical regions worldwide. General weed management measures such as manual plucking, fencing, and burning have shown limited effectiveness, largely due to associated health risks such as allergies, asthma, and hay fever (Navie *et al*., 1996; Bhowmik *et al*., 2007). Navie *et al*. (1996) also emphasized that hand pulling is ineffective unless followed by adequate disposal. Among the various control measures, biological control has emerged as the most effective approach for managing *P. hysterophorus* due to its low-cost, long-term sustainability, and environmental safety (Evans, 1997).

Zygogramma bicolorata Pallister (Coleoptera: Chrysomelidae) is a neotropical leaf beetle with characteristic yellow-brown striped elytra and is used in classical biological control due to its oligophagous leaf-feeding activity against *P. hysterophorus* (Dhileepan, 2001). Among nine insect species and one rust fungus imported to Queensland, Australia from Mexico as potential biocontrol agents for *P. hysterophorus*, *Z. bicolorata* was proved to be one of the most effective in controlling the weed (Dhileepan *et al*., 2000). Following the Australian experience, *Z. bicolorata* was introduced to Bangalore, India from Mexico between 1983 and 1984 (Jayanth, 1987). Although, there is no official record of its deliberate introduction into Nepal, the beetle was detected in August 2009 in Hetaunda (Makawanpur district, central Nepal), where it was identified as the primary agent responsible for the defoliation of Parthenium (Shrestha *et al*., 2010). It is probable that the beetle entered Nepal from India in the same way as *P. hysterophorus* due to the open border and free vehicle movement between India and Nepal (Shrestha *et al*., 2010).

Mature beetles and larvae of *Z. bicolorata* voraciously feed on *P. hysterophorus*, markedly suppressing the weed's growth, vigor, and seed production (Dhileepan *et al*., 2000). The beetle demonstrates a high degree of host specificity, with no reports of completing its life cycle on plant species other than *P. hysterophorus*. Although occasional feeding on sunflower leaves has been observed, the damage is minimal and typically occurs only when its pollen accumulates on the sunflower foliage (Ganga Vishalakshy *et al*., 2008). Rare instances of feeding on another invasive species, *Xanthium strumarium* L., have also been documented (Gupta *et al*., 2004; Shrestha *et al*., 2010). Therefore, *Z. bicolorata* poses minimal risk to native ecosystems or economically important crops in areas where *P. hysterophorus* is proliferating rapidly. In India, the natural spread of *Z. bicolorata* was found to be insufficient for effective control of *P. hysterophorus*, leading to large-scale mass rearing and targeted release programs to enhance its impact (Mahadevappa, 2009). In Nepal, where the beetle has been observed causing noticeable defoliation in some regions (Shrestha *et al*., 2010), detailed insights into its developmental biology, reproductive and feeding potential under local conditions remain scarce. Most prior studies are from other countries, which may not be directly applicable due to environmental and ecological differences. This creates a critical gap in understanding how this beetle performs under Nepalese conditions. Therefore, this study aims to systematically evaluate the life cycle and biocontrol performance of *Z. bicolorata* against *P. hysterophorus* under the greenhouse conditions of Lamjung, Nepal to inform future strategies for integrating classical biological control into invasive weed management programs in Nepal. The findings will provide baseline data necessary for future mass-rearing programs and integrated management strategies for controlling *P. hysterophorus* in Nepal.

MATERIALS AND METHODS

Experimental site

The research was conducted from May to August 2024 in the greenhouse and Entomology Laboratory of the Institute of Agriculture and Animal Science (IAAS), Lamjung Campus, located in Sundarbazar, Lamjung. The study site is situated at an elevation of 610 meters above sea level, with coordinates 28°07'54.8" N latitude and 84°25'11.7" E longitude, and is characterized by a humid subtropical climate. The research period was marked by typically warm and humid weather conditions. From May to August 2024, temperature and relative humidity were recorded daily inside the greenhouse. Maximum temperatures ranged between 34°C and 40°C, while minimum temperatures varied from 18°C to 23°C. Average relative humidity increased from 79.49% in early May to a peak of 82.78% in mid-July, remaining below 80% through August.

Rearing of beetles

Live insect specimens of *Z. bicolorata* were collected from the infested area around Sundarbazar, Lamjung. Fresh leaves of the parthenium plant was used for insect rearing. The collected larvae and beetles of *Z. bicolorata* was introduced in 3.5 kg capacity plastic jars covered with mesh lids. The wilted leaves were replaced daily with fresh ones. Newly hatched larvae were reared in separate jars and after maturity, they were transferred to plastic jars filled with moist soil for pupation. Following the subsequent stage, the adults were separated for the experiment.

Experimental setup to study the biology and life cycle of beetle

The biology and life cycle of the beetle were studied under laboratory conditions following the method described by Bajracharya *et al.* (2021). A pair of freshly emerged adult beetles were separated from stock culture and placed in a pot consisting parthenium plant comprising 32-33 leaves covered with nylon net. The beetles were allowed to mate and eggs were checked daily for hatching. Larvae were transferred into another pot after leaves were completely defoliated. Fully grown grubs were transferred to a plastic container (13 cm diameter* 30 cm) filled with moist sand for pupation. Completely randomized design (CRD) was followed and was replicated three times.

Table 1. Treatment combinations showing different plant growth stages and corresponding pairs of released beetles used in the experiment.

S. No.	Treatment	Stage of plant (S)	Pair of beetles (P)
1	S1P2	Seedling (6-8 leaves)	2
2	S1P3		3
3	S1P4		4
4	S1P5		5
5	S1P0		0
6	S2P2	Flowering (32-34 leaves)	2
7	S2P3		3
8	S2P4		4
9	S2P5		5
10	S2P0		0

'S' refers to the growth stage of *P. hysterophorus*: S1 represents the seedling stage with 6–8 leaves, and S2 represents the flowering stage with 32–34 leaves. 'P' refers to the number of *Z. bicolorata* pairs released per treatment. S1P0 and S2P0 represent the control treatments, with no beetle release.

Experimental setup to study feeding potential of beetles

The feeding potential of the beetles was studied following the method described by Jaiswal *et al.* (2023). A total of 10×3 i.e. 30 plastic pots of diameter and length of 15cm×5cm, respectively, were used for the experiment. 2.5 kg air-dried soil was transferred to each pot after mixing with farm-yard manure in the ratio of 2:2. Seedlings with 5-6 leaves were collected from the periphery of Sundarbazar, Lamjung and transplanted with two seedlings placed in each pot out of which the robust one was retained by thinning out the latter after successful establishment. Each pot was irrigated with equal quantity of water every three days and weeding was carried out manually whenever necessary. Each plot was enclosed with nylon nets (90 cm diameter and 140 cm height) of 2 mm mesh size to prevent beetle escape and to exclude other insects that could potentially harm the plant within the plots. A total of 10 treatments accommodating 2 stages of *P. hysterophorus*, viz., seedling stage (6-8 leaves) and flowering stage (32-34 leaves) with 0 (control), 2, 3, 4 and 5 pairs of *Z. bicolorata* beetles in each stage was utilized (Table 1). Each treatment along with control was replicated 3 times.

Data collection and analysis

The study involved measurement of key developmental and survival parameters across successive life stages of *Z. bicolorata*. Time intervals between successive stages were recorded at 24-hour increments. Morphometric measurements of eggs, larvae, pupae, and adults were obtained using calibrated Vernier calipers. Color changes of eggs, larvae, pupae, and adults was assessed visually to document phenotypic changes throughout development. The number of eggs laid per day and the count of empty eggshells were recorded manually to assess reproductive output and hatching success. Stage-specific survival rates were calculated as the percentage of larval emergence from total eggs, pupal recovery from total larvae, and adult emergence from total pupae. The feeding potential of *Z. bicolorata* was evaluated by monitoring its reproductive activity and the extent of foliar damage caused to the host plant. Variable densities of adult beetle pairs (2 to 5 pairs) were introduced onto *P. hysterophorus* at each of the seedling and flowering stages. The number of eggs laid by adult beetles and the number of larvae that successfully emerged were manually counted to determine reproductive performance and early-stage development. The duration required for complete defoliation was recorded in days to assess feeding intensity over time. Data obtained throughout the experiments were recorded in Microsoft Excel 2016. Analysis of parameters of feeding potential were carried out using R (ver. 4.3.3; Posit Team, 2024). We evaluated the significance of the comparisons with ANOVA and LSD test using agricolae package (ver. 1.3; de Mendiburu, 2023).

RESULTS AND DISCUSSION

Morphological features and lifecycle of *Z. bicolorata*

-The life cycle of *Z. bicolorata* comprises four distinct developmental stages: egg, larva, pupa, and adult as shown in Figure 1. The morphological characteristics, morphometric measurements, duration of developmental stages (Table 2), and reproductive performance (Table 3) across various life stages of the beetle are described in detail below.

Egg

Z. bicolorata laid its eggs either singly or in small clusters, typically on both the surface and under the leaves or stems of *P. hysterophorus*. The eggs were yellowish in color initially but turned reddish as they approached hatching. Under greenhouse conditions, the eggs generally hatched within 3 to 5 days. (Kumar *et al.*, 2023) reported an average incubation period of 4.6 days, ranging 5 to 6 days. Several researches reported an average incubation period of 4 days, consistent with previous findings (Pandey *et al.*, 2001; Mehta *et al.*, 2019). Each female beetle laid approximately 600-689 eggs during the lifetime. However, (Bajracharya *et al.*, 2021) reported a significantly higher average fecundity of 1,837 eggs per female under laboratory conditions at around 26±2°C and 70±10% relative humidity in Nepal while Mehta *et al.* (2019) reported an average of 663.5 eggs per female. The mean length and width of eggs were about 1.18±0.02 mm and 0.51±0.01mm, respectively. The mean percentage of egg hatch was 84.08 ±0.50%. Several studies reported that the color ranges from light yellow to yellowish-orange (Bajracharya *et al.*, 2021), occasionally orange, with a sub-shining surface turning slightly darker just before hatching (Kumar *et al.*, 2023).

Table 2. Result of reproductive and developmental parameters of *Z. bicolorata*.

S. No.	Parameters	Unit	Value	Range
1	Total number of egg laid	number/lifetime	650±21.45	600-689
2	Percentage of egg hatching	%	84.08±0.50	82-85
3	Percentage of pupa recovery	%	81.32±2.82	76-88
4	Percentage of adult production	%	78.69±0.88	77-80

Mean (± SE), standard error; n = 20 for each stage.

Table 3. Duration and morphometric measurements of developmental stages of *Z. bicolorata*.

S. No.	Stages	Time period (days)		Length (mm)		Width (mm)	
		Mean	Range	Mean	Range	Mean	Range
1	Egg	4±0.47	3-5	1.18±0.02	1.14-1.17	0.51±0.01	0.49-0.54
2	Larva						
	I instar	3.33±0.27	3-4	1.30±0.04	1.21-1.37	0.94±0.04	0.84-1.03
	II instar	4.00±0.47	3-5	2.96±0.07	2.81-3.1	1.21±0.04	1.11-1.29
	III instar	3.67±0.27	3-4	4.89±0.10	4.67-5.1	1.92±0.12	1.67-2.17
	IV instar	4.67±0.47	4-5	7.45±0.14	7.13-7.72	3.31±0.08	3.12-3.47
3	Pupa	11.33±0.54	10-12	5.78±0.07	5.65-5.93	3.74±0.07	3.61-3.89
4	Adult						
	Male	37.00±0.94	35-39	5.45±0.12	5.23-5.82	4.21±0.04	3.69-3.97
	Female	43.33±0.72	42-45	6.37±0.15	6.12-6.63	3.85±0.07	4.12-4.29

Mean (± SE), standard error; n = 20 for each stage.

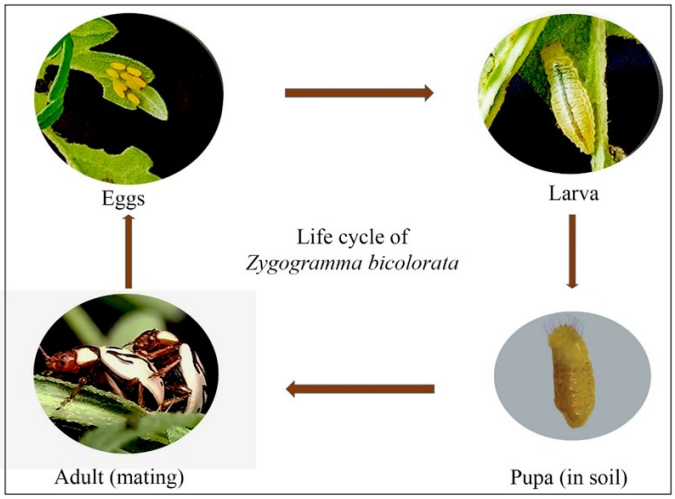

Figure 1. *Lifecycle of Z. bicolorata, showing four distinct stages: egg, larva, pupa, and adult.*

Larvae

The larval stage of *Z. bicolorata* consisted of four distinct instars, each marked by changes in color and size. Upon hatching, the neonate larvae were yellowish and gradually turned creamy white as they matured. This color change is consistent with descriptions by (Pawar & Korat, 2013) who noted the gradual shift in larval coloration during development. Morphometric measurements across instars show a consistent increase in size. For instance, the first instar averages 1.30 mm in length and 0.94 mm in breadth, progressing to the fourth instar, which measures approximately 7.45 mm in both length and breadth. These findings are in line with those reported by (Bajracharya *et al.*, 2021), who documented third and fourth instars measuring approximately 4.96 mm and 8.08 mm in length, respectively, under controlled laboratory conditions in Nepal. Similarly, Pawar & Korat (2013) reported slightly smaller dimensions, with fourth instar larvae reaching 6.25±0.12mm in length, indicating some variability based on environmental factors and rearing conditions. Behaviorally, the third instar was characterized by the voracious feeding, presence of spiracles on the thorax and abdomen of larvae, off-white appearance and covered by distinctly visible setae. These features are indicative of the larva's preparation for the final growth phase (Pawar & Korat, 2013). The size increased consistently to 4.89±0.10 mm and 1.92±0.12 mm, which surged up to 7.45±0.14 mm and 7.45±0.14 mm length and breadth, respectively, in the fourth instar. In the final stage, the larvae were more or less cylindrical in shape, with a prominent longitudinal line along their dorsal side characterized by sluggishness before entering pupation. Afaq *et al.* (2024) also emphasized the behavioral shift in mature larvae, highlighting how fully grown individuals ceased feeding and burrowed into the soil, often as deep as 10 cm, for pupation. The duration of each larval instar varies slightly but generally falls within a 3-5-day range, accounting a total larval period of approximately 13-18 days. This developmental timeline allows larvae to accumulate sufficient resources for successful pupation (Bajracharya *et al.*, 2021).

Pupa

After completing the larval stages, *Z. bicolorata* entered a pre-pupal phase, where it remained inactive for about 1-2 days before burrowing into the soil to pupate. About 81.32±2.82% of the larvae developed into pupae. During this stage, the larvae turned light yellow in color. Pupal chambers were formed by burrowing 3-5 cm into the soil, where they underwent metamorphosis. The pupal stage lasted approximately 10-12 days, after which the insect completed its development and emerged as an adult. The formation of pupal chambers at a depth of 3-5 cm provides mechanical protection and buffers against environmental extremes such as temperature fluctuations and desiccation. Jayanth & Bali (1994) observed similar behavior in Bangalore, India, where mature larvae burrowed into the soil to pupate at depths of 1-3 cm, remaining protected during their vulnerable metamorphic phase. The pupae measured approximately 5.65- 5.93 mm in length and 3.61- 3.89 mm in breadth. These measurements are comparable to those reported by

Bajracharya *et al.* (2021) reporting average pupal dimensions of 5.90 mm in length and 3.74 mm in breadth (Bajracharya *et al.*, 2021). Similarly, Mehta *et al.* (2019) recorded slightly larger pupae with a mean length of 6.24 ± 0.15 mm and breadth of 4.13 ± 0.13 mm during his experiments in India. These slight differences in pupal measurements across studies may be attributed to several ecological and physiological factors. Environmental conditions such as temperature and humidity, the nutritional status of host plants and the geographic variation in *Z. bicolorata* populations can significantly influence growth rates and ultimate body size. Warmer temperatures, for instance, have been linked to faster developmental rates but may result in slightly smaller body sizes due to shortened feeding periods during larval stages (Omkar *et al.*, 2008).

Adult

The average percentage of adult emergence from pupation was 78.69±0.88% which showed a slight decline from the pupa recovery percentage (81.32±2.82%). Adult emergence was recorded at 78.69±0.88%, indicating a relatively high conversion rate from pupa to adult stage. The adult *Z. bicolorata* beetle comprised a dull white to yellowish body covered with fine hairs which later displayed distinctive yellow to brownish coloration with dark, longitudinal stripes on their elytra. Males measured around 5.45±0.12 mm length and 4.21±0.04 width while females were slightly larger, reaching up to 6.37±0.15 mm and 3.85±0.07 mm length and breadth respectively. Adults fed actively on parthenium leaves, aiding in the biological control of this invasive weed. The life span of females (42-45 days) appeared to be longer than males (35-39) with the entire life cycle, from egg to adult, spanning roughly 62-78 days. Longer lifespan in females compared to males has similarly been observed by earlier researchers (Bajracharya *et al.*, 2021; Jayanth & Bali, 1994). In contrary, Siddhapara & Patel (2024) reported shorter lifespans for females (80.56 days) compared to males (85.48 days). This discrepancy might be attributed to differences in experimental setup, environmental factors such as temperature and humidity, or nutritional variations in host plant quality. Such contrasting results emphasize the importance of localized ecological and physiological influences on the biology of *Z. bicolorata*, and suggest that further investigations are needed to fully understand the sex-based longevity dynamics of this species.

Feeding potential of *Z. bicolorata*

Number of eggs deposited per plant

Adult beetles released on parthenium plants deposited eggs from the first day of introduction. As the number of beetles increased from 2 to 5 pairs, there was a clear upward trend in the number of eggs laid in both stages of plants as in Figure 2. In seedling stage, the highest number of eggs was recorded from the plants treated with 5 pairs (66.66) while the lowest was recorded from plants treated 2 pairs (30.33). Similarly, in flowering, the highest egg count was observed from plants treated with 5 pairs (184.33), with the lowest on plants treated with 2 pairs (120).

Number of larvae emerged per plant

The larvae of *Z. bicolorata* began to emerge after 3-4 days of exposure. *Z. bicolorata* larvae were recorded on all parthenium plants of flowering stage regardless of the number of beetle pairs used, with number of larvae emerged increasing as the number of beetle pairs increased from 2 to 5 as shown in Figure 2. The number of emerged larvae per plant treated with 5 pairs of beetles was highest (112.33), followed by those treated with 4 pairs (100.33) while lowest number of larvae emerged in plants treated with 2 pairs (68). However, in seedling stage, larvae were recorded only on plants treated with 2 and 3 pairs of beetles with 18.33 larvae emerging from plants treated with 3 pairs and 10 larvae from plants treated with 2 pairs.

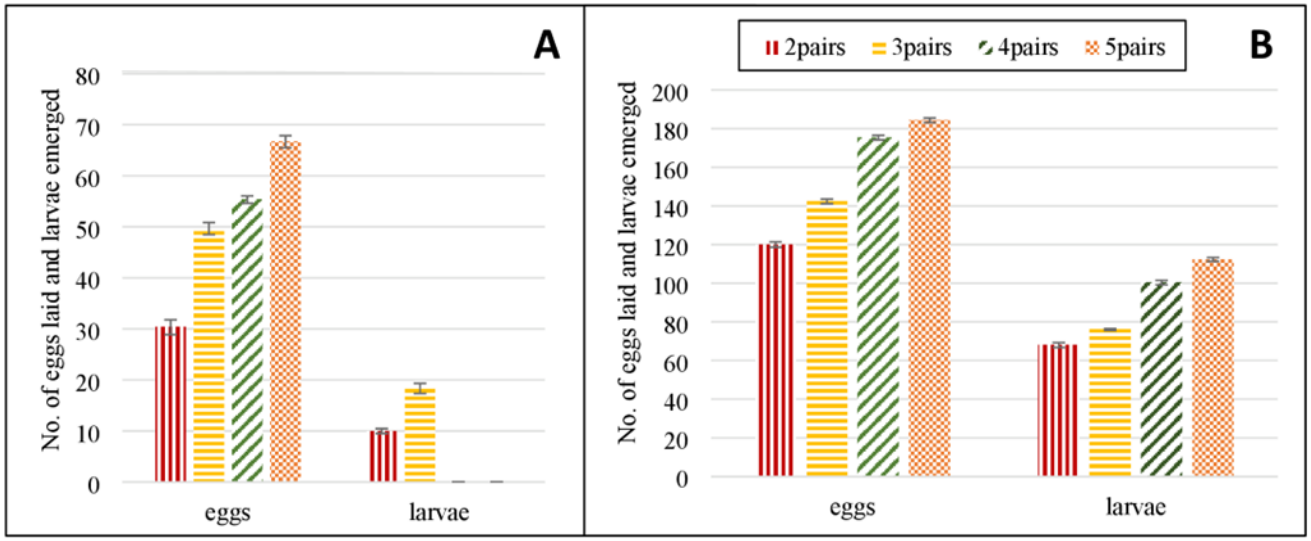

Figure 2. *Number of eggs deposited and larvae emerged on the P. hysterophorus in seedling stage (S_1) [A] and flowering stage (S_2) [B] treated with 2, 3, 4, and 5 pairs of Z. bicolorata.*

Table 4. Effect of different mating pairs of *Z. bicolorata* on egg deposition, larval emergence, and time required for complete defoliation of *P. hysterophorus* at seedling stage.

S. No.	Pairs of beetle	No. of eggs deposited	No. of larva emerged	Total days for complete defoliation
1.	2	30.33^{d}	10^{b}	6.66^{a}
2.	3	49.66^{c}	18.33^{a}	4^{b}
3.	4	55.33^{b}	0^{c}	2.33^{c}
4.	5	66.66^{a}	0^{c}	1.33^{c}
	Grand Mean	7.083	3.58	50.5
	SEM	1.42	0.665	0.408
	CV (%)	4.89	16.28	19.73
	LSD at 5%	4.65	2.17	1.33
	F-test	***	***	***

Values followed by the same letter within a column are not different based on LSD test (α = 0.05). *** indicates highly significant difference by F-test. SEM = standard error of the mean; CV = coefficient of variation; LSD = least significant difference.

Table 5. Effect of different mating pairs of *Z. bicolorata* on egg deposition, larval emergence, and time required for complete defoliation of *P. hysterophorus* at flowering stage.

S. No.	Pairs of beetles	No. of eggs deposited	No. of larva emerged	Total days for complete defoliation
1	2	120^{d}	68^{d}	15.66^{a}
2	3	142.33^{c}	76^{c}	13.66^{b}
3	4	175.33^{b}	100.33^{b}	9.66^{c}
4	5	184.33^{a}	112.33^{a}	6.66^{d}
	Grand Mean	155.5	89.16	11.41
	SEM	1.527	1.251	0.331
	CV (%)	1.70	2.43	5.03
	LSD at 5%	4.98	4.08	1.08
	F-test	***	***	***

Values followed by the same letter within a column are not different based on LSD test (α = 0.05). *** indicates highly significant difference by F-test. SEM = standard error of the mean; CV = coefficient of variation; LSD = least significant difference.

Time taken for complete defoliation

This study demonstrated that all released pairs of adult beetles defoliated the parthenium plant leaves. In seedling stage, plants treated with two pairs of beetles took the longest time for defoliation took the longest (6.67 days) while five pairs took the shortest (1.13 days) as in Table 4. Similarly, in flowering stage, defoliation took 15.67 days with two pairs and only 6.67 days with five pairs as represented in Table 5. The results are consistent with earlier study of Chandravanshi *et al.* (2018) where early-stage defoliation took 6.6 days with two pairs and 2.0 days with five pairs and in reproductive stage, defoliation times ranged from 14.33 to 15.33 days with two pairs and 5.0 to 5.33 days with five pairs. Similar findings were reported by Dhileepan *et al.* (2000) where defoliation led to a significant reduction in weed height, root and shoot biomass and seed production. Likewise, Chandravanshi *et al.* (2018) found that a higher beetle population (five pairs) caused more extensive leaf damage in a shorter period compared to lower populations (two pairs). Figure 3 illustrates the progressive defoliation and wilting damage caused by *Z. bicolorata* on *P. hysterophorus*, ranging from minor leaf nibbling (S1P2, S2P2) to severe foliage loss, stem exposure, and complete leaf skeletonization (S1P5, S2P5). In contrast, control plants (S1P0, S2P0) exhibit healthy, intact leaves with no visible damage. There is an inverse relationship between the number of beetle pairs used and the time taken for complete defoliation as shown in Figure 4. As the number of beetle pairs increased from 2 to 5, the time required for defoliation decreased significantly. This suggests that, higher population of beetle per plant is important for effective management of weeds. These findings align with those of Shabbir *et al.* (2016) and Kanagwa *et al.* (2020) who reported that beetle activity led to complete (100%) defoliation and marked decline in weed growth within 28 days. After the emergence of larvae, the leaves of the plants were more rapidly defoliated, indicating that larvae are voracious feeders and play a crucial role in weed control. Mohapatra *et al.* (2022) reported that larvae consume more foliage than adult beetles and recommended their use as a more effective management strategy for controlling this invasive weed.

Figure 3. *Progressive defoliation of P. hysterophorus by Z. bicolorata at two developmental stages: seedling (S_1) and flowering (S_2), illustrating the level and rate of damage under varying beetle pairs (P) released per plant. Diagrams depict plants at the earliest recorded time of complete defoliation: 1.13 days after beetle release in S_1P_5 and 6.67 days in S_2P_5.*

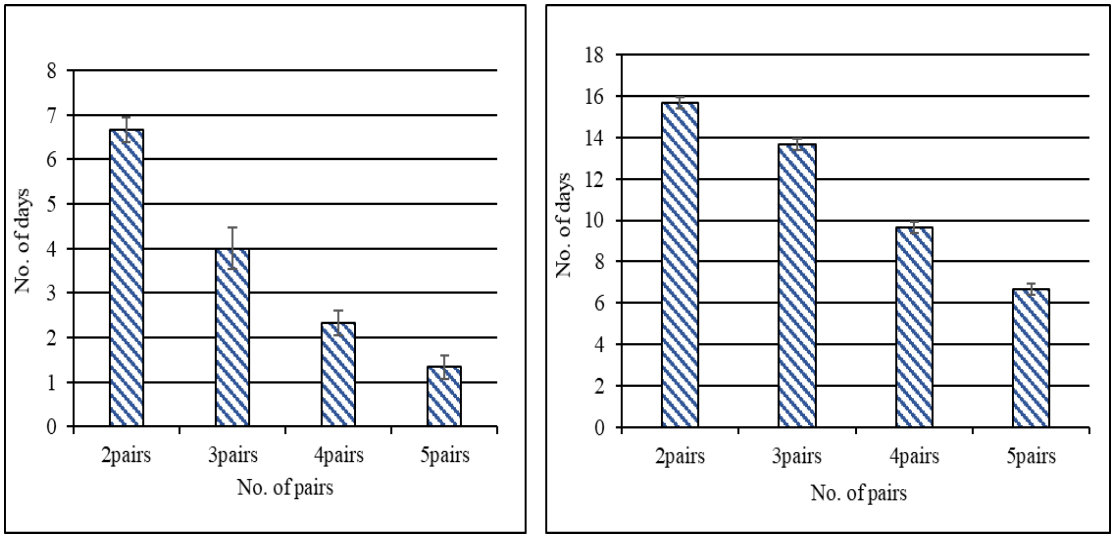

Figure 4. *Time taken for complete defoliation (in days) in seedling stage (S1) [A] and flowering stage (S2) [B] of P. hysterophorus treated with 2, 3, 4, and 5 pairs of Z. bicolorata.*

Conclusion

In conclusion, *P. hysterophorus* is a highly invasive weed that poses a serious threat to native plant species. *Z. bicolorata* has proven to be an effective biological control agent against this weed, significantly reducing its growth and development. The impact of *Z. bicolorata* on parthenium is more pronounced when defoliation was initiated at early stages of plant's growth. Moreover, the defoliation period tends to be shorter when a higher number of beetles were introduced. Therefore, deploying a greater number of beetle pairs can be advantageous for timely weed management, particularly before the plants reach their reproductive stage. As this study was conducted under greenhouse conditions, further research is required to validate under field settings to evaluate the beetle's establishment and effectiveness across the varied agro-ecological zones of Nepal.

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