

Impact of the leaf-feeding beetle *Zygogramma bicolorata* Pallister on the growth of *Parthenium hysterophorus* L. in climatically different locations of Nepal

Seerjana Maharjan^{1,2}, Bharat Babu Shrestha¹, Anjana Devktoa¹, Prakash Chandra Aryal³, Muniappan Rangaswami⁴, Pramod Kumar Jha^{1*}

¹Central Department of Botany, Tribhuvan University, Kirtipur, Kathmandu, Nepal

²Department of Plant Resources, Thapathali, Kathmandu, Nepal

³Goldengate International College, Tribhuvan University Affiliate, Kathmandu, Nepal

⁴IPM Innovation Lab, Virginia Tech, 526 Prices Fork Road, Blacksburg, VA 24061 USA

*CORRESPONDING AUTHOR:

Pramod Kumar Jha

Email:pkjhaprof@gmail.com

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ABSTRACT

The leaf-feeding beetle, *Zygogramma bicolorata* Pallister, one of the biocontrol agents against *Parthenium hysterophorus* L., having been established at several locations of Nepal. However, the damage to *P. hysterophorus* by the beetle has not been assessed quantitatively under different climatic conditions so far. The study was carried out at two locations, namely Hetauda with a tropical climate (540 m asl) and Kathmandu with a subtropical climate (1300 m asl) to evaluate the impact of the beetle on *P. hysterophorus*. Beetles were released in two different densities (one and two beetles/plant) during the early flowering period (early August). Individual plants were harvested when the initial signs of senescence were observed in mid-September. Plant height and leaf, stem, root biomass of each harvested individual was measured. Plant height, leaf biomass, and stem biomass were significantly higher at the subtropical site than at tropical sites and declined with the increasing density of beetle at both locations. Root:shoot ratio increased with the increasing density of beetle at both locations. The plant height, leaf biomass, and shoot biomass decreased up to 35 %, 80 %, and 54 % respectively at the tropical site and 9 %, 99 %, and 26 % respectively at the subtropical site. Root:shoot ratio increased by 75 % in the tropical site and 30 % in the subtropical site. The present study, therefore, suggests that mass-rearing and releasing of the beetle could be an effective management tool for the biological control of *P. hysterophorus* in tropical and subtropical climates of Nepal.

Keywords: Biocontrol, Herbivore, Invasive plant, Parthenium weed, Mexican beetle

1. INTRODUCTION

Parthenium hysterophorus L. (Asteraceae), commonly known as parthenium weed, is a native of tropical America and has been recognized as a major invasive weed in Asia's tropical and subtropical regions, Africa, and Oceania (Adkins *et al.* 2019). It has been categorized as a serious invasive species in approximately 49 tropical and subtropical countries globally (Navie *et al.* 1996; Adkins & Shabbir 2014; Bajwa *et al.* 2016; Maharjan *et al.* 2020). In Nepal, the weed was first reported during late 1960s, and currently, recognized as one of the most problematic invasive weeds with distribution from tropical lowland (Tarai) to Middle mountains with subtropical climate (Tiwari *et al.* 2005, Shrestha *et al.* 2019a). *P. hysterophorus* has altered soil properties and plant community structure (Timsina *et al.* 2011; Rokaya *et al.* 2020), reduced forage yield of grasslands (Shrestha *et al.* 2019b), and affected human and animal health (Shrestha *et al.* 2015, 2019b).

Among the various control measures of invasive species, biological control using arthropods, and pathogens is considered the most sustainable, environmental, and cost-effective approach (Jayanth 1987; McFadyen 1998; Dhileepan 2001; Barton *et al.* 2007; Strathie *et al.* 2011). Phytophagous biological control agents have been widely used with principal emphasis on controlling invasive plants in undisturbed areas such as rangelands, and these biological control agents have been established in most cases (Hajek 2004). The biological control of *P. hysterophorus* was first initiated in Australia in 1976, with 11 (9 insects and 2 rust) biocontrol agents having since been identified and released (Dhileepan & McFadyen 2012; Dhileepan *et al.* 2019).

Zygogramma bicolorata (Coleoptera: Chrysomelidae), one of the nine insect biological control agents of *P. hysterophorus*, is a native of tropical America, mainly the regions surrounding the Gulf of Mexico, and was imported to Australia for the first time in 1980 to manage *P. hysterophorus* (McFadyen and McClay 1981, Withers 1998, Dhileepan *et al.* 2019). It has been deliberately or unintentionally

introduced to many South Asian and African countries (India, Pakistan, Nepal, South Africa, Ethiopia, Tanzania and Uganda) and is now widespread and well established in Australia, India, Pakistan, and Nepal (Dhileepan *et al.* 2019, Kanagwa *et al.* 2020). The different life stages of *Z. bicolorata* – larvae and adults feed on the *P. hysterophorus* causing large-scale defoliation. In addition, *Z. bicolorata* is now one of the successful biocontrol agents due to higher fecundity and egg hatching success, a rapid increase in population once established (McClay 1985, Dhileepan *et al.* 2000a, 2000b). The beetles are host-specific, with both the larvae and adults feeding on the leaves and flowers of *P. hysterophorus* (Dhileepan *et al.* 2018). *Z. bicolorata* was first reported in Nepal in 2009, and now it is widespread across the entire country, where *P. hysterophorus* is found (Shrestha *et al.*, 2010, 2019a). Despite many studies in different countries related to *P. hysterophorus* and *Z. bicolorata* (Dhileepan *et al.* 2018), no study related to the impact of *Z. bicolorata* on *P. hysterophorus* has been conducted in Nepal. There was a report of bigger-sized *Z. bicolorata* with increasing elevation and decreasing temperature (Bhusal *et al.* 2019). *Z. bicolorata* was used in present study because this species was found to be one of the prominent biocontrol agents that mainly affects the aerial parts of *P. hysterophorus* (Dhileepan *et al.* 2000b) and is spreading in many parts of Nepal along with *P. hysterophorus* (Shrestha *et al.* 2019a). In addition, among two biological control agents, viz. *Z. bicolorata* and *Puccinia abrupta* var. *partheniicola* available in Nepal, *Z. bicolorata* is the most damaging one (Shrestha *et al.* 2015). Even though the growth of *Z. bicolorata* varies with climatic conditions, it is unclear how the damage incurred by *Z. bicolorata* on *P. hysterophorus* varies in different climatic conditions. Thus, plant feeding experiments was carried out by using *Z. bicolorata* in two localities having different climatic conditions of Nepal. The primary aim of this study was to investigate the effect of herbivory by the *Z. bicolorata* on the growth of *P. hysterophorus* in two locations in central Nepal having a tropical and subtropical climate. The results of this study could be useful

for selecting localities to release *Z. bicolorata* on *P. hysterophorus* infested areas in Nepal.

2. MATERIALS AND METHODS

2.1 Experimental Sites

To determine the effect of *Z. bicolorata* feeding on the growth of *P. hysterophorus*, the field experiment sites in two climatically different localities, Brindaban, Hetauda in Makwanpur district (27.42°N, 85.01°E, 540m asl) and Shorakhutte in Kathmandu district (27.72°N, 85.31°E, 1297m asl) were selected (Figure 1).

Hetauda has a tropical and humid and Kathmandu has a sub-tropical climate. *P. hysterophorus* has invaded roadside vegetation and grasslands at both sites, displacing many native species (Karki 2009; Timsina *et al.* 2011). The average annual rainfall for 10 years (2007-2016) is 2200 mm and 1400 mm for Hetauda (Hetauda N.F.I station, a nearby station of the experimental plot) and Kathmandu (Panipokhari station, a nearby station of the experimental plot) respectively. The average monthly maximum temperature was the highest (35°C) in April, and the minimum temperature was the lowest (8°C) in January in Hetauda. The average monthly maximum temperature was the highest (31°C) in June, and the minimum temperature was the lowest (5°C) in January in Kathmandu (DHM 2016).

2.2 Seed Collection

To prepare the seedlings for the experiment, seeds were randomly collected from *P. hysterophorus* population in Lamsure Danda (27.41°N, 85.02°E, 527m asl), Hetauda and Kirtipur (27.67°N, 85.29°E, 1292m asl), Kathmandu (Figure 1) during mid-August, 2016 then placed in respective single paper bags. They were air-dried under shade for 2 to 3 days, then stored at 4°C in a refrigerator (Baskin & Baskin 2014) prior to the experiment.

2.3 Preparation of *P. hysterophorus* Seedling and Experimental Plots

P. hysterophorus nursery of 2 m × 2 m was established at both experimental sites to prepare seedlings. The nurseries were prepared by plowing

the land in open space with partial shade during late May 2017. After all the plant-debris were removed, 500-600 *P. hysterophorus* seeds were spread into the nursery. Nurseries were watered regularly to maintain the soil moist. A total of nine 1 m × 1 m experimental plots were prepared in each site. Adjacent plots were 0.5 m apart. Before starting the experiment, experimental plots were ploughed up to 10 cm depth and all the plant traces were removed manually. Two weeks later, sixteen seedlings with similar heights were transferred to each 1 m × 1 m experimental plot from the nursery. The distance kept between the two seedlings was 20 cm. Each plot was covered with nylon nets (100 cm × 100 cm × 200 cm) to prevent the beetles from escaping and avoiding the entry of other insects that may damage *P. hysterophorus* inside the plots.

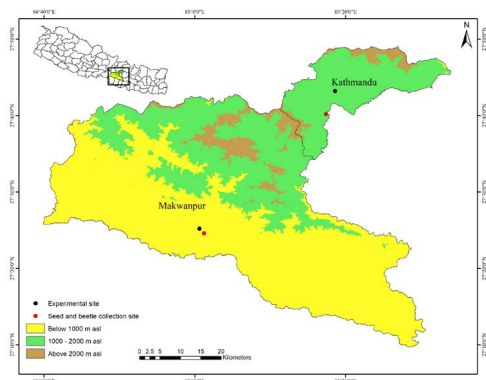


Figure 1: Map showing experimental sites in Hetauda (Tropical region) and Kathmandu (Subtropical region)

2.4 Collection and Release of *Z. bicolorata*

To create a balance between male and female individuals (Figure 2), adult *Z. bicolorata* in pairs were hand picked from the same sites from where seeds were previously collected on August 3, 2017, from Hetauda and August 6, 2017, from Kathmandu. Three separate ventilated plastic bottles (12 cm diameter and 6 cm depth) were used to collect eight pairs of beetles and another three plastic bottles were used to collect sixteen pairs of beetles. Fresh leaves of *P. hysterophorus* were kept inside the plastic bottle until they were

released from the beetle collection site to the experimental plots. Thereafter, collected adult *Z. bicolorata* were released into caged experimental plots simultaneously on the same day in two experimental sites (on August 3 at Hetauda and August 6 at Kathmandu). The treatments were arranged in a Complete Randomized Design (CRD) with three treatments – control (0) with no beetles; Treatment-1 (one beetle per plant in the ratio 1:1, i.e., eight pairs of beetles per plot) and Treatment-2 (two beetles per plant in the ratio 2:1, i.e., 16 pairs of beetles per plot). All three treatments were replicated three times.

The plots were monitored regularly for four months (mid-June to mid-September) until the

end of the experiment. The constant feeding of *P. hysterophorus* by *Z. bicolorata* inside the plot were checked at the regular interval maintaining the position of the nylon nets. At the end of the growing season, after six weeks of the introduction of beetles, during mid-September all *P. hysterophorus* individuals were uprooted. Before harvesting, the height of each individual was measured above the ground level to the terminal portion using measuring tape. The stem, leaf, and root portion of the individual plant was packed in a separate paper bag and oven-dried (70°C for 48 hours). The oven-dried biomass of each sample was measured using a digital balance (0.001g).



Figure 2: *Zygogramma bicolorata* collected in pair for field experiment

2.5 Soil Nutrient Analysis

The soil samples were collected from the depth of 0-10 cm from the center of each plot during mid-September as the root of *P. hysterophorus* generally reached up to 10 cm depth. Soil samples were dried in the shade for a week and later tested for soil nitrogen (micro Kjeldahl method following Horneck & Miller 1998), soil phosphorus (Olsen's method following Gupta 2000), and soil potassium (Flame photometer method following Trivedi & Goel 1986).

2.6 Data Analysis

Data were explored to assess the data quality and properties using descriptive statistics and visualization. All the variables were not normally

distributed. A generalized linear model (GLM) was used incorporating the Gamma family with a 'log' link, as dependent variables were always positive with the right-skewed distribution. Graphical diagnostics were used via package DHARMA (Florian 2020), which also tests simulated residuals using Kolmogorov–Smirnov test (KS test) and dispersion test where no significant issues were found. Graphs were prepared using ggplot2 (Wickham 2016), and all the analyses were carried out in R program version 3.6.1 (R Core Team 2020).

3. RESULTS AND DISCUSSION

3.1 Variation in Plant Height and Biomass

Plant height, stem, leaf, and root biomass of *P. hysterophorus* in all the treatments were found

to be higher at the subtropical experimental sites than at tropical, whereas root:shoot ratio was higher at the tropical sites than at the subtropical (Table 1, Figure 3). Treatment-2

(32 beetles/m²) resulted in almost complete defoliation due to the feeding by *Z. bicolorata* in some plots of the tropical sites as compared to the control plot.

Table 1: Mean values of the parameters studied with the range values in two localities (0 – control, 1 – treatment-1 (one beetle per plant), and 2 – treatment-2 (two beetles per plant))

Parameter	Treatment	Tropical site		Subtropical site	
		Mean±SD	Range	Mean±SD	Range
Plant height (cm)	0	130±27	75-195	190±28	104-242
	1	114±31	35-175	183±19.5	144-219
	2	84±31	20-150	173±23	100-211
Stem biomass (g)	0	11.9±8.4	2.43-41.46	27.9±20.4	1.68-89.49
	1	10.2±7.9	0.57-42.8	23.4±12.3	3.76-47.86
	2	5.4±4.5	0.21-20.78	20.7±14.2	3.61-64.34
Leaf biomass (g)	0	2.3±1.6	0.18-7.28	7.4±5.3	0.91-24.83
	1	1.7±1.6	0.07-7.75	4.3±2.4	0.64-10.83
	2	0.6±0.6	0-2.53	3.1±2.8	0.15-12.26
Root:shoot ratio (%)	0	8.1±2.6	2.67-14.96	8.3±1.7	5.49-13.44
	1	9.5±3.7	2.63-25.67	7.7±2.4	0.07-13.32
	2	14.2±4.8	6.87-28.47	10.8±4.6	5.14-24.76

Higher biomass and taller plants in subtropical sites could be due to higher soil nutrients in subtropical sites than in tropical sites. Higher soil nutrients (nitrogen, phosphorus and potassium) in the experimental plots of the subtropical site than in tropical sites (Figure 4) suggest that the growth of *P. hysterophorus* could be better in subtropical sites than in tropical sites. Rainfall and temperature of the subtropical site are comparatively lower than that of the tropical site (DHM 2016). In addition, the polluted environment in subtropical sites with more atmospheric CO₂ compared to the tropical site (Pokhrel *et al.* 2018) might have favored the more vigorous growth of *P. hysterophorus*, as elevated CO₂ enhances *P. hysterophorus* growth as found in India (Pandey *et al.* 2003).

It has been pointed out that the performance of the beetle is mainly governed by temperature and rainfall (Dhileepan 2003; Omkar & Pandey, 2008). Jayanth & Bali (1993) reported that the temperature around 20-30°C is suitable for the growth and survival of the *Z. bicolorata*. Slightly high temperatures and more rainfall in tropical

sites (Max. temp – 29.7°C, Min. temp – 17.3°C and annual rainfall approx. 2200 mm) compared to the subtropical site (Max. temp – 27.4°C, Min. temp – 14.2°C and annual rainfall approx. 1400 mm) (DHM 2016) might be the reason for better performance of the beetle in the tropical site, and thus there was more damage to *P. hysterophorus*. In addition, a greater reduction in plant height and total biomass at the tropical site might be because of the low capacity of *P. hysterophorus* individuals to produce new leaves after previously produced leaves were damaged by the beetles due to the low soil nutrients.

3.2 Effect of Locality and Treatment on Plant Height and Biomass

Variation in plant height, leaf biomass, stem biomass, and root:shoot ratio among localities and treatments showed that the effectiveness of herbivory varies between localities (Figure 3). The plot level average plant height, leaf biomass, and stem biomass were higher for the subtropical site than the tropical site (Figure 3).

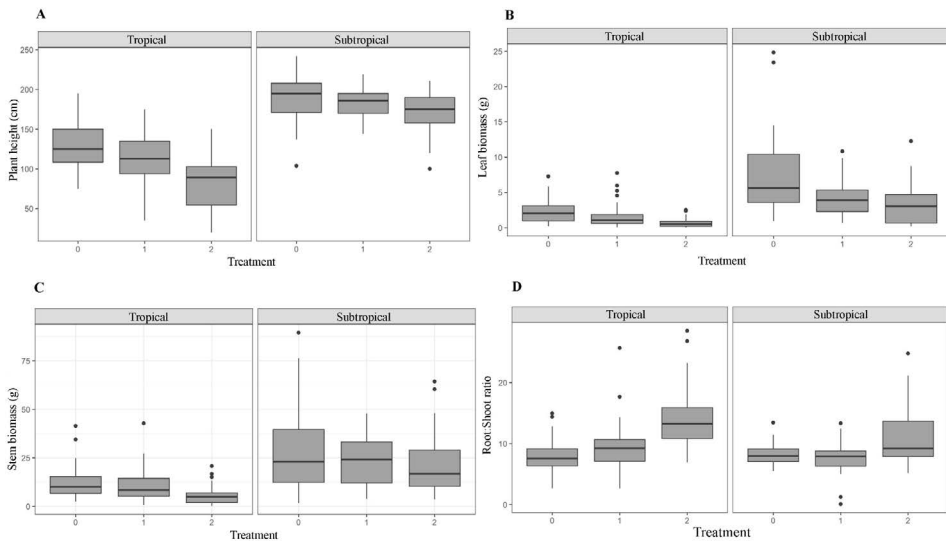


Figure 3: Variation in plant height (A), leaf biomass (B), Stem biomass (C), and root:shoot ratio (D) among localities.

Note: 0 – control, 1 – Treatment-1 (One beetle per plant), 2 – Treatment-2 (Two beetles per plant)

Plant height, leaf biomass, and stem biomass were significantly reduced by *Z. bicolorata* in both sites. A decrease in leaf, stem biomass, and height with increasing beetle density was found (Figure 3). The decrease in plant height was significant for both treatments in tropical sites, whereas only Treatment-2 (two beetles per plant) in the subtropical site

was significant from control (Table 2). In tropical sites, plant height decreased by 11 % in Treatment-1 (one beetle per plant) and 35 % in Treatment-2 (two beetles per plant) compared to control. Whereas, in the subtropical site, Treatment-2 (two beetles per plant) showed a significant decrease in height by 9 % compared to control (Table 2, Figure 3A).

Table 2: Summary of a generalized linear model expressing the relation of different parameters with treatments for two locations

Parameter	Site	Coefficient	Estimate	Std.Err.	t value	p-value	Lo. CI	Up. CI
Plant height (cm)	Tropical	Intercept	4.86	0.04	113.04	< 2e-16	4.78	4.94
		Treatment1	-0.12	0.06	-2.1	0.037	-0.24	-0.008
		Treatment2	-0.43	0.06	-7.17	3.96E-11	-0.55	-0.31
	Subtropical	Intercept	5.24	0.019	269.18	< 2e-16	5.21	5.28
		Treatment1	-0.038	0.027	-1.37	0.171	-0.09	0.016
		Treatment2	-0.094	0.027	-3.48	0.001	-0.14	-0.041
Leaf biomass (g)	Tropical	Intercept	2.26	0.29	7.71	2.43E-12	1.77	2.95
		Treatment1	-0.6	0.36	-1.66	0.099	-1.37	0.09
		Treatment2	-1.6	0.31	-5.21	6.75E-7	-2.31	-1.07
	Subtropical	Intercept	7.42	0.81	9.21	4.87E-16	6.04	9.26
		Treatment1	-3.15	0.93	-3.38	0.001	-5.16	-1.41
		Treatment2	-4.28	0.87	-4.92	2.40E-6	-6.21	-2.72

Stem bio-mass (g)	Tropical	Intercept	2.47	0.11	21.82	< 2e-16	2.25	2.71
		Treatment1	-0.14	0.15	-0.92	0.35	-0.45	0.16
		Treatment2	-0.78	0.15	-4.92	2.39E-6	-1.09	-0.47
	Subtropical	Intercept	3.32	0.09	34.44	<2e-16	3.14	3.52
		Treatment1	-0.17	0.13	-1.29	0.19	-0.44	0.09
		Treatment2	-0.29	0.13	-2.22	0.02	-0.56	-0.03
Root:shoot ratio (%)	Tropical	Intercept	2.08	0.05	40.44	< 2e-16	1.98	2.18
		Treatment1	0.16	0.072	2.23	0.026	0.019	0.302
		Treatment2	0.56	0.072	7.84E+0	10.00E-13	0.42	0.71
	Subtropical	Intercept	2.12	0.048	43.69	< 2e-16	2.022	2.21
		Treatment1	-0.07	0.068	-1.12	0.263	-0.212	0.057
		Treatment2	0.26	0.067	3.92	0.000	0.132	0.39

Note: Generalized linear model (GLM) was performed using Gamma family with 'log' link, multiplicative. Texts shown in bold are significant at $p < 0.05$

The release of two beetles per plant showed a significant decrease in leaf biomass by about 80 % for the tropical site and by about 99 % in the subtropical site compared to controls. Additionally, the release of one beetle per plant in the subtropical site also showed a significant reduction in leaf biomass, a reduction by 96 % compared to the respective control (Table 2, Figure 3B). Similarly, treatment-1 (one beetle per plant) did not result in a significant reduction in stem biomass for both sites, but treatment-2 (two beetles per plant) resulted in a significant reduction in stem biomass by 54 % for the tropical site and a reduction of 25 % for subtropical site compared to respective controls (Table 2, Figure 3C).

The reduction in biomass and plant height in the current study is similar to other studies showing that above ground biomass or height of the weeds generally decline in response to biological control agents/insects (Dhileepan 2001; Thomas & Reid 2007). Studies on the pre-flowering release of beetles resulted in the reduction in plant height by 35% under experimental conditions (Dhileepan *et al.* 2000b) and a slightly high reduction in plant height (18-65%) and plant biomass (55-89%) due to defoliation caused by Mexican beetles on *P. hysterophorus* in the field condition in Australia (Dhileepan *et al.* 2000a). Hence, if any herbivore partially or fully suppresses the invasive weed, such weed may be easily outcompeted by other

native plant species (Hajek 2004; Shabbir *et al.* 2013, 2018). The use of biocontrol agents is thus effective in managing noxious and invasive weeds such as *P. hysterophorus*.

The release of two beetles per plant resulted in an overall increase in root:shoot ratios for both localities (Figure 3D), but the release of one beetle per plant showed a significant increase in root:shoot ratio by 17 % the tropical site. The root:shoot ratio significantly increased by 75 % in the tropical site and by 30 % in the subtropical site in the plot containing two beetles per plant (Table 2). More reduction in aboveground biomass due to feeding resulted in the increased root:shoot ratio in tropical site. The increased herbivory resulted in an increase in the root:shoot ratio at both sites. An increase in root:shoot ratio due to herbivory was also reported in *Rumex obtusifolius* (Bentley & Whittaker 1979). *Z. bicolorata* feeds on the aerial part of *P. hysterophorus*, thereby reducing shoot mass. Hence, the root:shoot ratio became higher with increased defoliation in the plants (Dhileepan *et al.* 2000b). In addition, the allocation of the parts is greatly affected by the timing of (Prins & Verkaar 1992). Defoliation subjected to the early flowering stage instead of the rosette stage by the introduction of *Z. bicolorata* might be the reason that there is no allocation of resources from root to stem to compensate the resources hence increasing the root:shoot ratio (Dhileepan

et al. 2000b). Soil nutrient analysis showed that soil nitrogen, soil phosphorus, and soil potassium were higher in subtropical sites than in tropical sites (Figure 4).

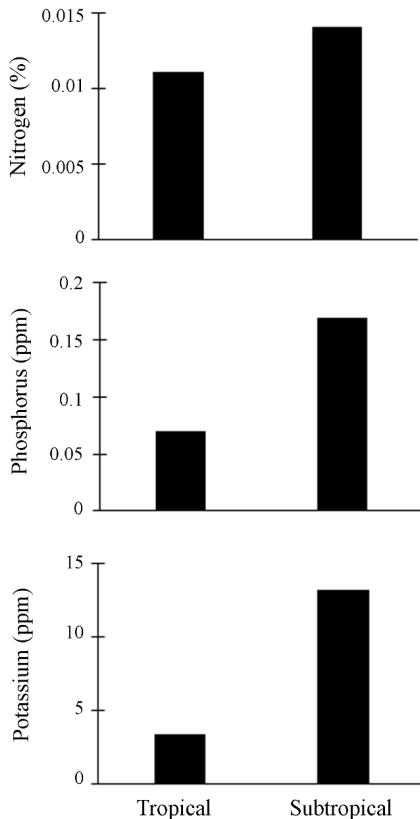


Figure 4: Soil nutrients in two different study localities

P. hysterophorus has been observed as a major threat to agriculture and the environment for the past few decades in Nepal. It has harmed plant species diversity and changed soil properties and production of crops and livestock (Tiwari *et al.* 2005; Timsina *et al.* 2011; Shrestha *et al.* 2015; Shrestha 2019). Still, in many parts of the country, local people, environmental managers, and governmental organizations are not well informed or prepared for management (Shrestha *et al.* 2015). The present study clearly showed the possibility of damage caused by *Z. bicolorata* to *P. hysterophorus* in areas having both tropical and subtropical climates. Hence, mass rearing and releasing of the

beetle would be a highly effective management tool for the control of *P. hysterophorus* in these two climatic zones of Nepal. As the use of biological control agents for *P. hysterophorus* found to be effective in the present study and previous studies conducted by many workers (Dhileepan *et al.* 2000a, 2000b; Ditomaso 2000, Kelton & Price 2009; Adkins & Shabbir 2014), the finding could be useful for the formulation of proper management plans in the future.

This study has illustrated the effectiveness of *Z. bicolorata* in two climatically different localities of Nepal in controlling *P. hysterophorus* through field experiments. Hence, the studies on the impact of *Z. bicolorata* on the growth of *P. hysterophorus* should be carried out in other tropical and subtropical parts of Nepal by mass-rearing and releasing the beetle to manage the weed. The studies on redistribution and regular monitoring of *Z. bicolorata* in climatically suitable areas where *P. hysterophorus* is causing major impacts should be carried out in the future. In addition to the management possibilities of *P. hysterophorus* using *Z. bicolorata*, the present findings can be helpful to enhance the growth of ecologically important native plants by reducing the invasiveness of *P. hysterophorus*.

4. CONCLUSION

Parthenium hysterophorus is a major agricultural and environmental threat in Nepal. The weed has caused harm to native plant species, crop yields, livestock, and altered soil properties. Among two fortuitous biological control agents available in Nepal, the Mexican leaf-feeding beetle, *Zygogramma bicolorata*, is the more widespread and most damaging. We studied plant feeding experiments using *Z. bicolorata* in two different localities of Nepal, having tropical and subtropical climatic conditions. *Z. bicolorata* significantly reduced the leaf biomass of *P. hysterophorus* by 80 % in tropical and 99 % in subtropical site. The present findings could be used for the mass rearing and releasing of the beetle in the *P. hysterophorus* infested areas with tropical and subtropical climatic conditions and could be used to manage *P. hysterophorus* in Nepal properly.

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