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Research article

Ecological parameters and biotic damage pattern in invasive alien species across land-use types in Kathmandu, Nepal

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Abstract

Invasive alien plant species (IAPS) are responsible for severe damage to ecosystems, causing huge economic loss. It has been considered that the IAPS are comparatively less damaged by herbivores and pathogens in the invaded range. This study assessed the effects of herbivore and pathogen damage (biotic damage) on the common IAPS, *Ageratina adenophora*, *Ageratum conyzoides*, *Bidens pilosa*, and *Parthenium hysterophorus* in the Chobhar area of Kathmandu, Nepal. Additionally, the density, frequency, and coverage of these IAPS were compared across different land-use types, such as fallow land, forest, and roadside, along the northern and southern aspects. A negative relationship was observed between *B. pilosa* and *A. adenophora*, as well as *P. hysterophorus*, suggesting competition for light resources, where one species reduced the cover of the other. Biotic damage differed across land-use types, with *B. pilosa* experiencing less damage in forest and *P. hysterophorus* more damage along road verges, indicating habitat-specific activities of herbivores and pathogens.

Keywords: Plant invasion, herbivores, pathogens, damage assessment, land-use type.

Introduction

Invasive alien plant species (IAPS) are rapidly spreading worldwide. They are among the main drivers of ecosystem change in their introduced range (Hejda *et al.* 2017; Rai and Singh 2020; Tallamy *et al.* 2021). Such ecosystem changes include alterations in soil properties, nutrient availability, water and light regimes, and declining biodiversity and productivity (Bellard *et al.* 2016; Downey and Richardson 2016).

IAPs are well-known for their ability to harm native plants by outcompeting them for resources such as light, water, and nutrients (Gallardo *et al.* 2016; Knauf *et al.* 2021). Most of the studies are focused on the impact of IAPS on native species richness, composition, abundance, seed germination, and seedling development (Thapa *et al.* 2017; Darji *et al.* 2021; Chhogyel *et al.* 2021). However, when two or more IAPS are present together, the dynamics of their interaction may become more complex. It is important to consider whether they facilitate each other's spread or compete with each other and with native species. These relationships can also alter ecosystem functioning and nutrient cycling by making the invaded habitat more hostile for the survival of native plants (Belote *et al.* 2006). Understanding how multiple IAPS interact is therefore crucial for predicting their cumulative impact on native ecosystems. In this context, field assessments, even basic surveys of IAPS abundance and cover, can provide useful insight into their relationship.

The success of IAPS can be attributed to several factors, which includes their competitiveness, adaptability, and allelopathy that allow the IAPS to outcompete native plants (Zheng *et al.* 2015; Yuan *et al.* 2021; Poudel *et al.* 2024). Another factor contributing to their spread and dominance in the invaded sites is the low level of damage from natural enemies, such as herbivores, pests, or pathogens (Hajek and Eilenberg 2018). With low damage, the IAPS enable them to grow faster and spread more aggressively than native species, which often experience serious damage by their enemies (Myers and Sarfraz 2017).

In Nepal, *Ageratina adenophora* (Spreng.) R.M. King & H. Rob., *Ageratum conyzoides* L., *Bidens pilosa* L., and *Parthenium hysterophorus* L. are the major IAPS across different ecosystems such as road verges, fallow lands, forest areas, and agroecosystems (Shrestha *et al.* 2022; Bhatta *et al.* 2024). In some cases, natural enemies of these IAPS have arrived in Nepal and are contributing to their damage. For example, *Zygogramma bicolorata* Pallister (a biocontrol agent of *Parthenium hysterophorus*) and *Procecidochares utili* Stone (a biocontrol agent of *Ageratina adenophora*) have spread to Nepal (Shrestha *et al.* 2019; Sharma Poudel *et al.* 2020; Pathak *et al.* 2021).

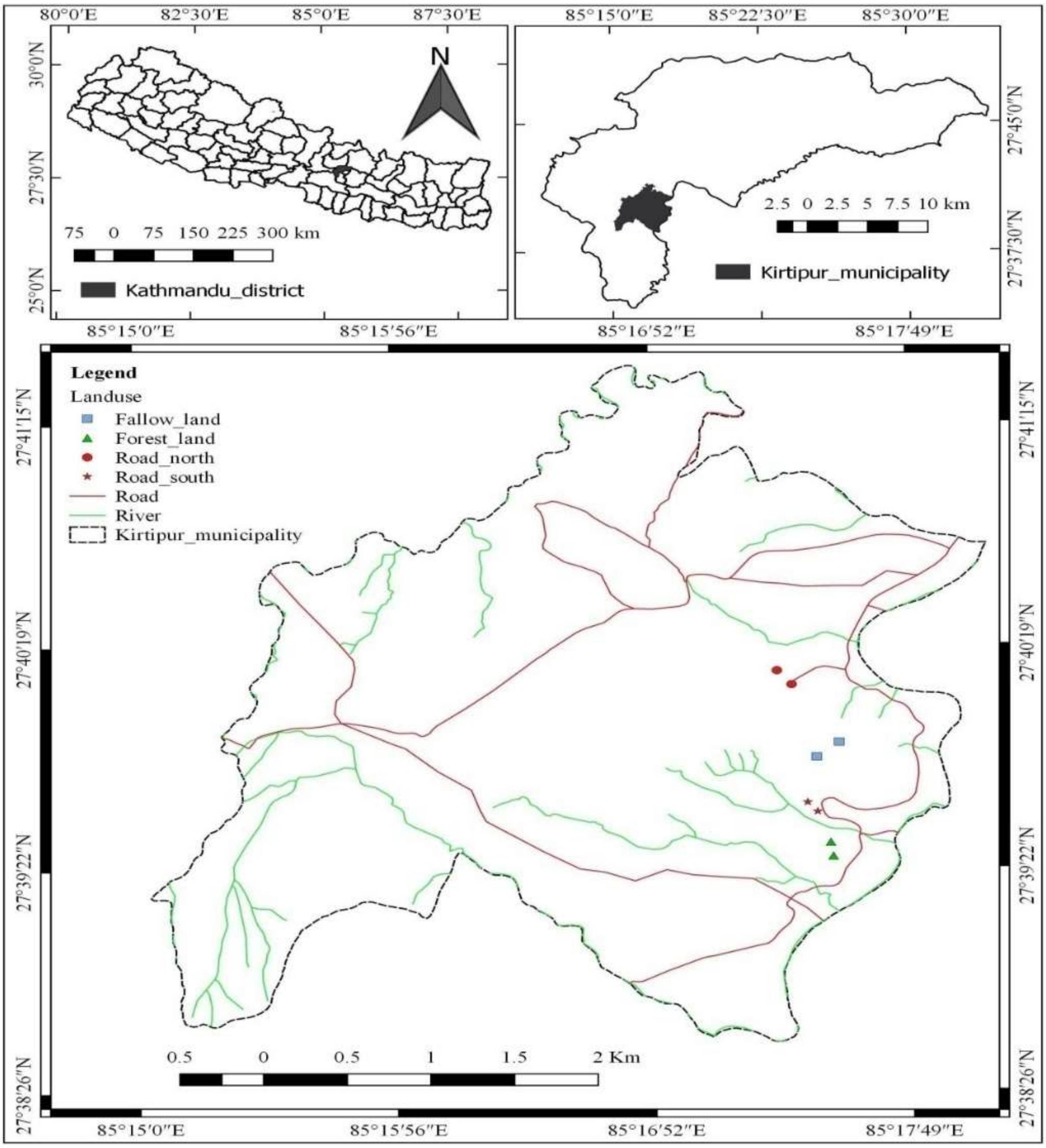

Figure 1. Study site, clockwise from top left: (a) Nepal map showing Kathmandu District, (b) Kathmandu District showing Kirtipur Municipality, and (c) Kirtipur Municipality showing study sites.

However, their effectiveness remains poorly understood. Additionally, since the invasion history of these IAPS spans several decades (e.g., *A. adenophora* was first reported in Nepal in 1958; Tiwari *et al*. 2005), it is also possible that the generalist herbivores might have started to feed on these IAPS (Bezemer *et al*. 2014). This possibility also remains unexplored. Studies examining whether such herbivores have developed associations with the IAPS and the extent of damage they cause are equally important for identifying effective biological control agents.

Before assessing particular herbivores associated with specific IAPS, it is important to first determine whether biotic damage is occurring. Additionally, it is equally important to evaluate the extent of invasion in a particular habitat by measuring key ecological parameters, including the interaction among the IAPS in the same invaded habitats. Understanding of such interactions will have significance in determining whether the damage observed is influenced by the competition between invasive plants themselves or by the habitat type where they have invaded. Considering these factors, this study aims to analyze the biotic damage and the relationship among IAPS.

Materials and methods

STUDY SITE

The study was conducted at Chobhar (27°27'07" N and 85°28'08' E and 1310 to 1346 m asl.) of Kirtipur Municipality in Kathmandu district, Bagmati Province, Nepal (Figure 1). It is famous for a gorge, ancient settlements, recreational sites with some fallow lands and patches of forest. The study area is characterized by mean minimum and maximum temperatures of 11.70°C and 24.76°C, respectively, with an annual rainfall of 1425 mm (worldweather.wmo.int).

Four land-use types (forest, fallow land, road verges towards north and south-facing slopes) were studied in Chobhar. Pine forests lying towards the north and south aspects were heavily invaded by *Ageratina adenophora* and *Bidens pilosa*. The roadside towards the north aspect was invaded by *Ageratum conyzoides*, *Bidens pilosa,* and *Parthenium hysterophorus*. The roadside on the south aspect was invaded by *Parthenium hysterophorus*, *Bidens pilosa*, *Ageratum conyzoides,* and *Ageratina adenophora*. The fallow land facing a southern aspect was invaded mainly by *Parthenium hysterophorus* and *Xanthium strumarium* L.

ECOLOGICAL ASSESSMENT OF IAPS

The occurrence of IAPS was recorded by sampling the quadrats of size 1×1 m^2 at each land-use type along transects. In the forest and fallow land, five transects (100 m long) were made, and in each transect, 5 quadrats were sampled. A total of 25 quadrats were sampled at each land-use type, where the numbers of individuals of each species were counted as density (Thapa *et al.* 2020). Based on the species occurrence in the plots, their frequency was also calculated. The percentage cover of each IAPS present in each quadrat was visually estimated by assessing the proportion of ground area occupied by its canopy (Kent 2011).

BIOTIC DAMAGE ASSESSMENT

Biotic damage assessment was conducted for each IAPS found in the sampled plots. Three individual plants of each IAPS were selected randomly in each quadrat, and the branches and leaves of IAPS damaged by herbivores and pathogens were counted. The percentage of damaged branches and leaves was calculated. In the case of *Ageratina adenophora*, individual shoots were considered instead of branches. The damage percentage refers to the proportion of branches or leaves that show any level of visible symptoms of insect bites, leaf spots, leaf rolling and curling with other deformations, galls or tumors, and blights, rather than the proportion of tissue that is completely destroyed. For each plant, the total number of branches or leaves was counted, and the number exhibiting these symptoms was recorded.

$$\text{Biotic damage (\%)} = \frac{\text{No. of branches or leaves damaged}}{\text{Total no. of branches/leaves present}} \times 100$$

STATISTICAL ANALYSIS

Simple linear regression was used to show the relationship between IAPS density and cover values. Biotic damages on the branches of *Bidens pilosa* were analyzed using one-way analysis of variance (ANOVA) to compare among the sites. In the case of *Ageratina adenophora*, *Ageratum conyzoides,* and *Parthenium hysteronphorus*, biotic damages were compared using an

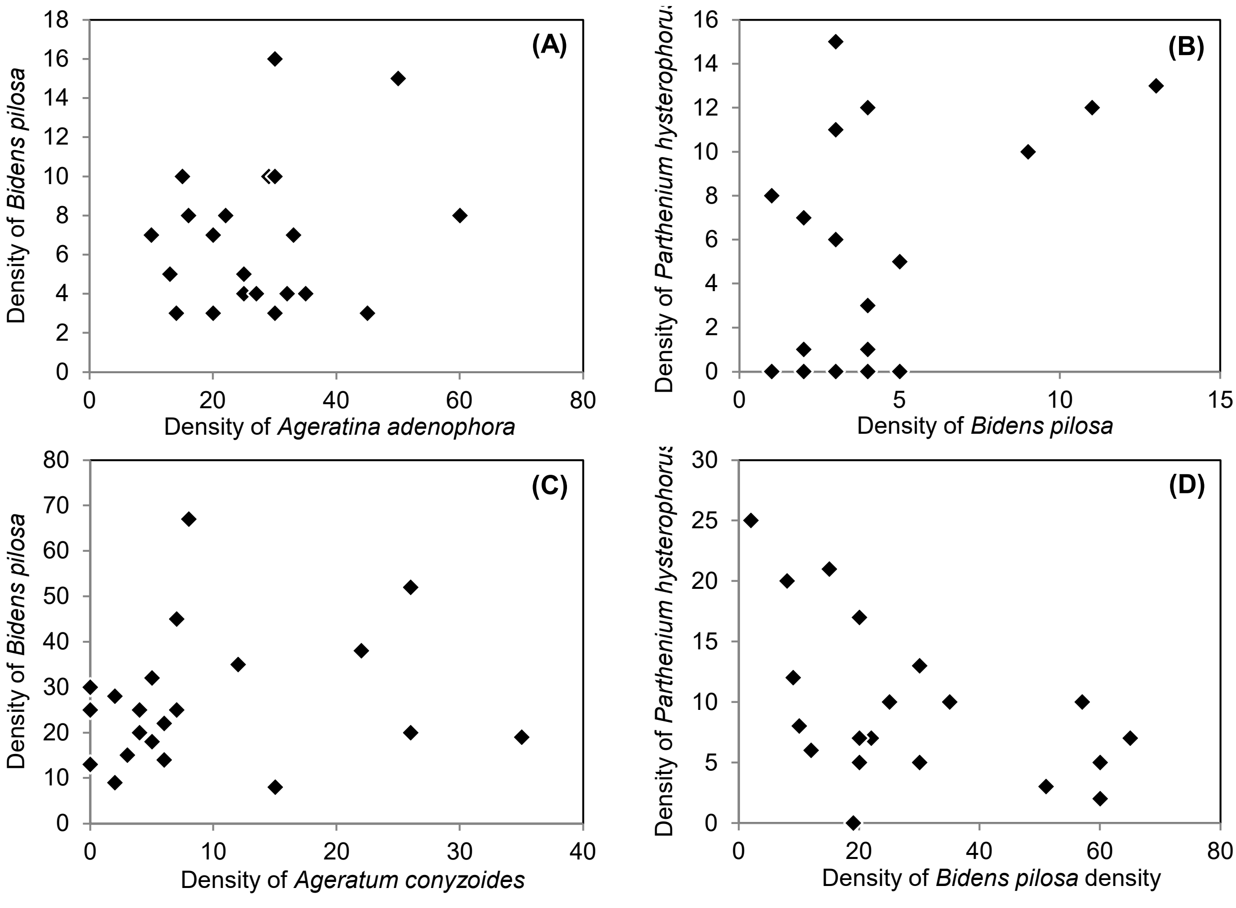

Figure 2. Relationships between the density of *Bidens pilosa* and the densities of (A) *Ageratina adenophora* in the forest, (B) *Parthenium hysterophorus* in fallow land, (C) *Ageratum conyzoides* in the road towards the northern aspect, and (D) *Parthenium hysterophorus* in the road towards the southern aspect.

independent sample *t*-test between two land-use types. The Kruskal-Wallis test was used to compare the damage in leaves of *Bidens pilosa* among the land use types, and the Mann-Whitney U test was used to compare the damage in branches of *A. adenophora* and *A. conyzoides* at two sites, as the data did not show normal distribution. All analyses were carried out using the Statistical Package for Social Science (SPSS, version 23) (IBM Crops. 2015).

Results

DENSITY AND COVER-WISE RELATIONSHIPS AMONG INVASIVE ALIEN PLANT SPECIES

Regression analysis showed that there was no significant relationship of *Bidens pilosa* density with the density of *Ageratina adenophora* in the forest (p = 0.822) and with *Parthenium hysterophorus* in fallow land (p = 0.131) (Figure 2). Along the roadside towards the north aspect, the relationship between *Bidens pilosa* and *Ageratum conyzoides* densities was insignificant (p = 0.446). A similar result was found on comparing the densities of *Bidens pilosa* and *Parthenium hysterophorus* along the road towards the southern aspect (p = 0.124) (Figure 2).

There was a negative relationship between the cover of *Bidens pilosa* and *Ageratina adenophora*. The cover of *B. pilosa* decreased with increasing the cover of *A. adenophora* (p = 0.020, Figure 3). In fallow land, there was no positive or negative relationship between the cover values of *B. pilosa* and *Parthenium hysterophorus* (p = 0.0764). Similarly, no significant relationship was found between the covers of *B. pilosa* and *Ageratum conyzoides* (p = 0.101). Along the roadside towards the southern aspect, the cover of *P. hysterophorus* decreased with increasing cover of *B. pilosa* (p = 0.004, Figure 3).

BIOTIC DAMAGES

Counting the branches, the biotic damage in *Bidens pilosa* was the least in the forest compared to road sides and fallow land (p < 0.001, Figure 4A, Table 1). Similarly, the number of damaged leaves in *B. pilosa* was higher in plants along the road verges towards the north and in the fallow land than that of the road

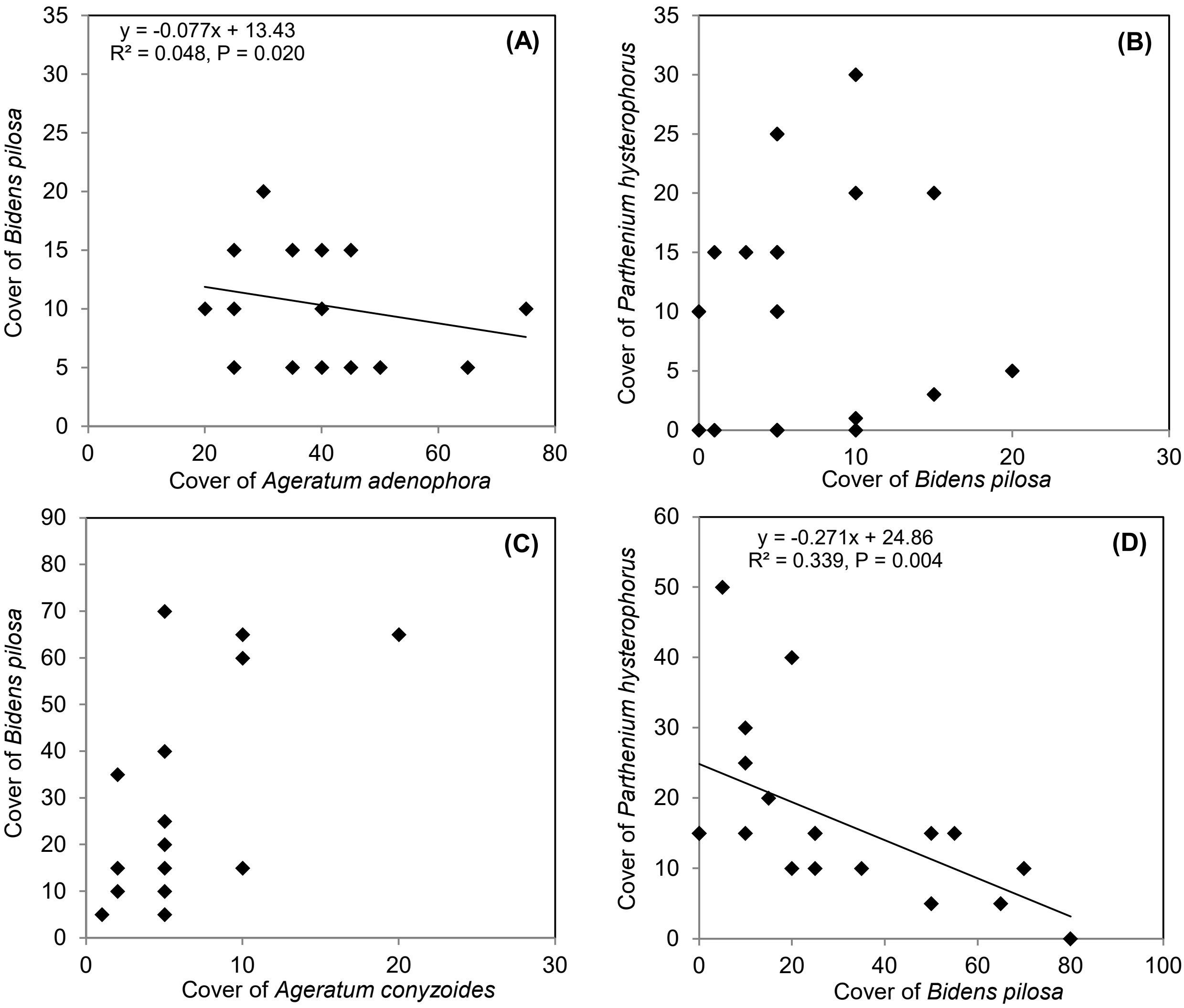

Figure 3. Relationships between the covers of (A) *Bidens pilosa* and *Ageratina adenophora* in the forest, (B) *B. pilosa* and *Parthenium hysterophorus* in fallow land, (C) *Ageratum conyzoides* and *B. pilosa* in the roadside towards the north aspect, and (D) *B. pilosa* and *P. hysterophorus* in the roadside towards the south aspect.

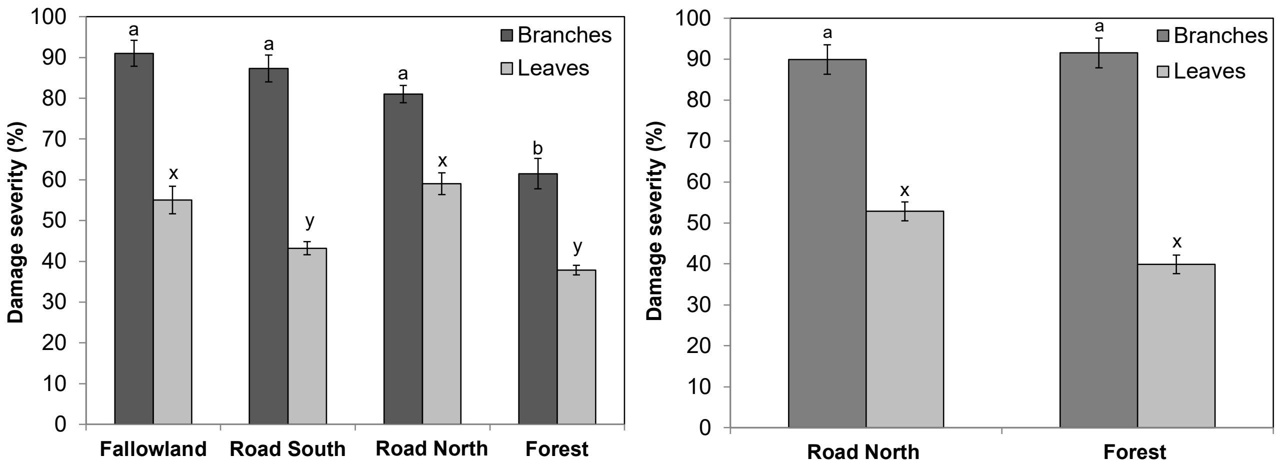

Figure 4. Biotic damages in *Bidens pilosa* (left) and *Ageratina adenophora* (right). The letters above the error bar 'a-b' indicate significant differences in branches and the letters 'x-y' in leaves among the land-use types (based on one-way ANOVA for branches and Kruskal-Wallis Test for leaves in *Bidens pilosa*; Man-Whitney U test for branches and independent sample t-test for leaves in *Ageratina adenophora*).

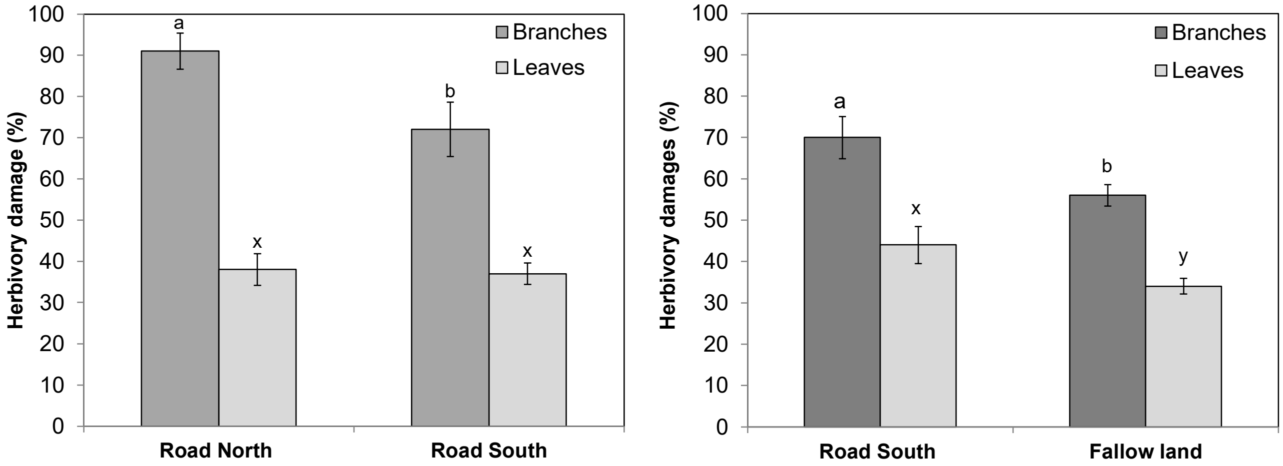

Figure 5. Biotic damages in *Ageratum conyzoides* (left) and *Parthenium hysterophorus* (right). The letters above the error bar 'a-b' indicate significant differences in branches, and the letters 'x-y' in leaves between land-use types (based on the Man-Whitney *U* test for branches and independent sample *t*-test for leaves).

Table 1. Results of statistical analyses on damaged branches and leaves of the invasive alien plant species studied.

Species	Plant parts compared and test methods used	Test statistic	Df	*P*- value
Bidens pilosa	Branches (one-way ANOVA)	*F* = 15.758	3	< 0.001
	Leaves (Kruskal-Wallis Test)	X^2 = 37.081	3	< 0.001
Ageratina adenophora	Branches (Mann-Whitney U test)	*U* = 0.999	42	0.780
	Leaves (t-test)	*t* = 3.406	42	0.785
Ageratum conyzoides	Branches (Mann-Whitney U test)	*U* = 13.405	42	0.002
	Leaves (t-test)	*t* = 3.864	42	0.266
Parthenium hysterophorus	Branches (t-test)	*t* = 21.389	42	0.001
	Leaves (t-test)	*t* = 8.111	42	0.007

towards the south and forest ($p < 0.001$; Table 1). There was no difference in biotic damage on *Ageratina adenophora* between road verges (north aspect) and forest ($p > 0.005$, Figure 4, Table 1).

Ageratum conyzoides was found in road verges (north and south aspects). In other sites, data were insufficient and therefore not used in this analysis. In this species, the highest number of biotic damage in the branches was found along the road on the north aspects than on the south aspects ($p = 0.002$, Figure 5, Table 1). Similarly, the number of damaged leaves was also higher along the road on the north aspect than on the south aspect; however, the result was statistically insignificant ($p = 0.266$, Figure 5, Table 1).

In the case of *Parthenium hysterophorus*, the number of branches showing damage symptoms was found to be high along the roadside towards the south aspect (70%) compared to the fallow land ($p = 0.001$, Figure 5, Table 1). Similarly, the number of damaged leaves was higher in the roadside towards the south aspect (42%) than that of fallow land (35%) ($p = 0.007$, Figure 5, Table 1).

Discussion

The study sites included the road verges, fallow land, and forest (pine forest). These were the IAPS-invaded sites. Results showed that there was no significant relationship between the density of *Bidens pilosa* and the densities of *Ageratina adenophora*, *Ageratum conyzoides,* and *Parthenium hysterophorus* (Figure 2). The lack of relationships indicates that the densities of these species are mostly independent under the conditions of the study sites.

Comparing the cover of *Bidens pilosa* with that of *Ageratina adenophora,* we observed a significant negative relationship, suggesting potential competition where higher cover of one species reduces the cover of the other. Similarly, the negative association between the cover of *B. pilosa* and *Parthenium hysterophorus* also indicates competitive interactions along the road towards the south aspect (Figure 3). However, the lack of a significant relationship in fallow land between *B. pilosa* and *P. hysterophorus* implies reduced competition, allowing both species to coexist without affecting each other's coverage.

The results show the effects of varying microclimatic conditions. Previously, the density, frequency, and cover of these IAPS across land-use types were highlighted (Thapa *et al.* 2020), while the current analysis examines the relationship among these parameters for the two species. It is important to note that the relationship between densities and cover does not necessarily reflect direct ecological interactions. However, these patterns indicate the responses of the IAPS to environmental factors, habitat conditions, and disturbance regimes (Yang *et al.* 2020). Previous studies have shown that all these IAPS reduce native plant density, species composition, and diversity (Timsina *et al.* 2011; Thapa *et al.* 2020; Sharma *et al.* 2023). However, both their effects on native species and the relationship among their own densities and covers appear to be site-specific, varying with local environmental conditions (Matter 2000).

Plants compete for light when one species' canopy shades another, reducing its access to sunlight and hindering growth (Valladares *et al.* 2016). The results suggest that the IAPS outcompete for light resources, leading to a decrease in *Bidens pilosa* cover due to high cover of *Ageratina adenophora* or a decrease in *Parthenium hysterophorus* cover due to increased cover of *B. pilosa.* Additionally, without accounting for the density and cover of other associated native species, attributing variation in the abundance of two species to direct interactions may not be justifiable. However, in the present study, only a few scattered small herbs, such as *Cynodon dactylon*, *Centella asiatica*, and *Oplismenus* sp., were present, and their abundance was negligible. Given this extremely low diversity, the influence of these minor species is expected to be minimal, and any competitive dynamics are likely to occur primarily between the IAPS themselves.

Branches and leaf damage studies suggest that the herbivory or pathogen activity in these species depends on habitat type and location. Counting the branches and leaves, the biotic damage was low in *Bidens pilosa* in the forest compared to the road verges and fallow land. In *Ageratina adenophora*, a greater number of branches were damaged on the northern side, and in the case of *Parthenium hysterophorus*, damaged branches and leaves were greater in the roadside compared to fallow land (Figure 4). In *Bidens pilosa*, the lower biotic damage in the forest is likely due to the effect of the forest canopy, where a shaded environment might have negatively affected the herbivore abundance or activities. Moreover, the diversity of herbaceous plants and shrubs might have supported more herbivores in the fallow land (Kavana *et al.* 2019). For *Parthenium hysterophorus*, the high level of biotic damage on road verges compared to fallow land could be attributed to the disturbance, as well as the ease of mobility for herbivores along the road corridors.

A beetle, *Zygogramma bicolorata*, a natural enemy of *Parthenium hysterophorus*, has been introduced to Nepal, which is contributing to some degree of damage in the weed (Shrestha *et al.* 2019). Similarly, a gall fly (*Procecidochares utilis*), a natural enemy of *Ageratina adenophora,* has its population in Nepal, but its damage has been reported to be less significant (Sharma Poudel *et al.* 2020). These insects were found in the study sites; therefore, it can be expected that these might have damaged their respective hosts.

In the present study, damages to both the leaves and branches of *Parthenium hysterophorus*, *Ageratina adenophora,* and *Bidens pilosa* were observed. Results highlighted that the biotic damage ranged from 40 to 100% depending on the plant species and microclimatic conditions. The damage percentage measured in this study does not refer to the complete destruction of the plant tissues. Rather, it reflects the number of branches or leaves that showed visible signs of biotic (herbivore or pathogen) damage. A leaf or branch was recorded as 'damaged' even if there was a sign or a small portion of damage. Thus, the proportion may reach 100% biotic damage if all of the leaves or branches of plants exhibited some level of symptom, not because they were fully consumed or severely damaged.

Additionally, native herbivores in Nepal, which could be adapted to these IAPS, may also have contributed to the damage. Therefore, understanding the full extent of biotic damage and damage severity across different environmental conditions is crucial for evaluating the effectiveness of biocontrol strategies.

Such studies will also help to determine whether introducing additional herbivores/pathogens or the native herbivores/ pathogens could enhance the management of IAPS.

Overall, the relationships between the cover of two IAPS and biotic damage on them vary across land-use types and aspects. It gives insight into habitat-specific herbivores or pathogen activity and potential interspecific competition. Hence, the study highlights the need for site-specific management of IAPS and understanding the impacts of biotic agents. Identifying potential biotic agents is crucial for evaluating and enhancing biocontrol efforts against such IAPS in Nepal.

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