Effects of Slope Aspect on *Ageratina adenophora* (Spreng.) King & H. Rob. Density and Galls Formed by Its Natural Enemy *Procecidochares utilis* Stone, 1947 in Makwanpur, Nepal

Seeta Pathak[1], Tej Bahadur Darji[1], Reetu Deuba[1], Gunanand Pant[2], Ramesh Raj Pant[3] & Lal B Thapa[1*]

[1]Central Department of Botany, Tribhuvan University, Kathmandu, Nepal
[2]Department of Biology, Kailali Multiple Campus, Kailali, Nepal
[3]Central Department of Environmental Science, Tribhuvan University, Kathmandu, Nepal
* Email: lal.thapa@cdbtu.edu.np

Abstract

A forest killer plant, *Ageratina adenophora* (Spreng.) King & H. Rob., has become a highly problematic weed in Nepal. Its control and management is a challenging issue. Studies on different factors that impact on its invasiveness are still deficient. On the other hand, assessment of its biological control agent, the *Procecidochares utilis* Stone, 1947 in Nepal remains untouched. This study aims to analyze the impacts of slope aspect on *A. adenophora* density and the galls formed by *P. utilis* in Central Nepal. The study revealed that the slope aspect influences the *A. adenophora* plant density, number of gall-bearing plants and gall density. These parameters were the highest in the south-east facing slope comparing to the north-west facing slope indicating that the infestation by *P. utilis* depends on the aspects. The findings of this study will have significance in developing management strategies for *A. adenophora* with the application of *P. utilis* as a biological control agent in Nepal.

Keyword: Biocontrol agent, Biological control, Forest killer plant, Gall fly, Invasive alien species, Kalo banmara

Introduction

Ageratina adenophora (Spreng.) King & H. Rob., commonly called the '*forest killer plant*' or '*Kalo Banmara*' in Nepali, is one of the naturalized invasive alien species in Nepal (Tiwari et al., 2005). It is a member of the family Asteraceae and a perennial herbaceous plant having a height of about 1-3 m. It has severely colonized different habitats such as fallow lands, agroecosystems, roadsides, degraded areas and forests (Tiwari et al., 2005; Thapa et al., 2015; Poudel et al., 2019). Its distribution range is tropical to lower temperate region throughout the country (Shrestha, 2016). It has been recognized as a noxious invasive alien plant throughout the world as it has wide negative impacts on the native ecosystems. For example, it can alter soil physicochemical and biological properties, and has broader negative impacts on native biodiversity (Tiwari et al., 2005; Balami et al., 2017, 2019; Poudel et al., 2019; Thapa et al., 2017; 2020a; 2020b).

Several eco-geographical factors such as elevation, native species diversity and distribution, canopies of trees, disturbances and soil moisture are reported as the factors affecting distribution and abundance of *A. adenophora* (Thapa et al., 2016; Yang et al., 2017). However, information on the relationship between biotic/abiotic factors and *A. adenophora* invasiveness is still limited. On the other hand, the control and management of this plant is a challenging issue (Wan et al., 2010). Its minute and easily dispersible seeds, long-term seed bank in the soil, fast germination and growth rate, vegetative mode of reproduction and good adaptability in varied conditions of soil and habitats are the key characteristics responsible for its rapid colonization in novel range (Wan et al., 2010). Several methods have been proposed and implemented in different parts of the world for its control and management. Several physical, chemical and biological methods of control and management of *A. adenophora* are recommended (Poudel et al., 2019). Among them, biological control using *Procecidochares utilis* Stone, 2047, a natural enemy of this plant, has been

proven to be one of the most promising methods, as this method is not only natural and environment-friendly, but also cost-effective.

P. utilis (Diptera: Trypetidae), an insect, is a natural enemy of *A. adenophora* found in its native range (Heystek et al., 2011). It forms galls in the stem of the plant and therefore it is called as the gall fly. The larvae of the insect grow inside the gall and due to the galls, the plants become stunted, the number of flowers and seeds are reduced, and ultimately, plants may even die (Bennett & Van Staden, 1986). The environmental and invasion biologists have given much attention to the application of *P. utilis* as the biological control agent of *A. adenophora.* This insect has been introduced to many countries such as New Zealand, Australia, South Africa, China etc. (Kluge, 1991). Successful control of *A. adenophora* by this agent has been reported from Hawaii, USA but the effective results from other countries including India and China are yet to be confirmed (Buccellato et al., 2012; Yang et al., 2017).

In the context of Nepal, Balami & Thapa (2017) have initiated damage assessment in leaves of *A. adenophora* and compared the damage with native *A. nepalensis* but they have not focused on the damage caused by the *P. utilis* galls. Shrestha (2016) highlighted that *P. utilis* has spread all over Nepal but its damage level is insignificant. Some of the studies showed that the elevation gradient (Poudel et al., 2020) and moisture conditions (Goeden, 1978; Li et al., 2006) affect the galls forming activities. The in-depth investigation regarding other factors, which have positive or negative impacts on the galls, is yet to be explored. The knowledge of such factors affecting *P. utilis* and its galls in *A. adenophora* could have a great contribution to the application strategies of biological control agents. In this study, we have investigated whether there are effects of slope aspect on the density of *A. adenophora* and the galls formed by *P. utilis* in an invaded community forest of Makwanpur district, Bagmati Province, Nepal.

Materials and Methods

Study site

This study was conducted in Takhtar Community Forest at Thaha Municipality-9, Chitlang of Makwanpur district (Bagmati Province), Nepal (Figure 1). The forest (27°24’59.99"N and 85°01’60.00"E, elevation 1750 to 1900 m asl) lies behind the famous Chandragiri hill about 22 km from the capital city Kathmandu towards the south-western part of Kathmandu valley. The community forest covers an area of about 158 ha and expands from south-east to north-west slope. Each of the slope aspects of the forest is disturbed by anthropic activities such as lopping, trampling and grazing. Both the slope aspects are therefore heavily invaded by invasive *A. adenophora* (personal observation).

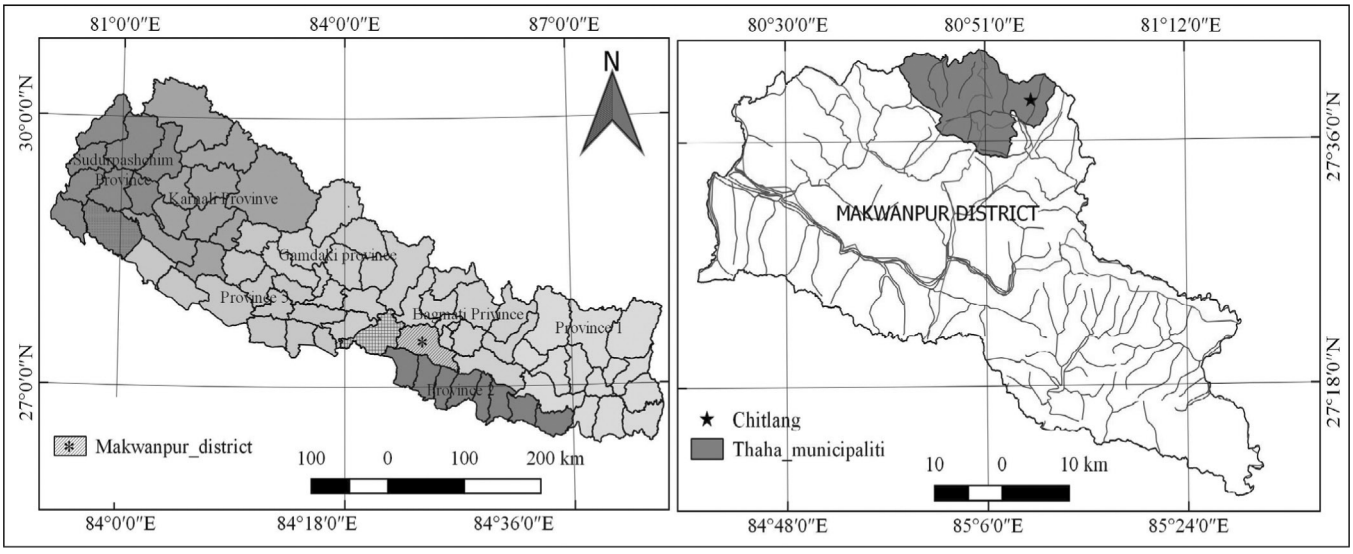

Figure 1: Study area map, Makwanpur district, Bagmati Province, Nepal.

The north-east slope was denser in terms of number of trees and the canopy gap was lesser than the south-east slope. The lower belt of the forest was the mixed type where *Morella esculenta* (Buch.-Ham. ex D. Don) I. M. Turner, *Schima wallichii* (DC.) Korth., *Lyonia ovalifolia* (Wall.) Drude, *Berberis aristata* DC., *Rubus ellipticus* Smith, *Viburnum* spp. were the major plant species. The upper belt of the forest was dominated by Pine trees.

Quadrat sampling

A total of 40 quadrats of size 1×1 m^2 each were sampled in the *A. adenophora* invaded sites of the community forest. Out of 40 quadrats, 20 quadrats were sampled at each of south-east and north-west aspects of the forest. In each aspect, five parallel transects of length about 20 m each were laid and in each transect, 4 quadrats were sampled. The length of each transect was 20 m and the distance between the two adjacent transects was 10 m. The distance between the two adjacent quadrats was at least 5 m.

Gall assessment

In each quadrat sampled, the number of individual plants of *A. adenophora* (erect stems from rootstocks) was counted as the density per plot. The number of plants having galls formed by *P. utilis* was counted from each plot. The number of galls per plot and per plant were also calculated.

Statistical analysis

The densities of *A. adenophora,* numbers of gall bearing plants per quadrat and the numbers of galls per plant in south-east and north-west facing slopes of the study site were compared using independent sample t-test. Mann-Whitney U-test was applied to compare the total number of galls per quadrat as the data were not normally distributed. The software R (version 4.0.3) was used for the analysis. The *p*-value <0.05 was considered for the significant differences in the mean value.

Results and Discussion

The density of *A. adenophora* in the south-east facing slope of the Takhtar Community Forest was greater than in the north-west facing slope. The number of *A. adenophora* in the south-east slope was 79.45±5.34 individuals/quadrat while the number was 57.55±3.54 individuals/quadrat in the north-west facing slope (t = 3.414, df = 38, p = 0.001, Figure 2).

The densities of gall bearing individuals of *A. adenophora* (number of gall bearing plants/quadrat) in the south-east and north-west facing slopes differed significantly. In the south-east facing slope, the number of gall-bearing plants was the highest i.e. 12.2±1.45 plants/quadrat whereas there were 6.6±0.57 gall bearing plants/quadrat in the north-west facing slope (t = 3.595, df = 38, p < 0.001, Figure 3). The gall-bearing plants in the south-east aspect was 15.36±1.64% while in the north-east aspect the gall-bearing plants were 11.47±1.62% (df = 38, t = 1.51, p = 0.056; Table 1).

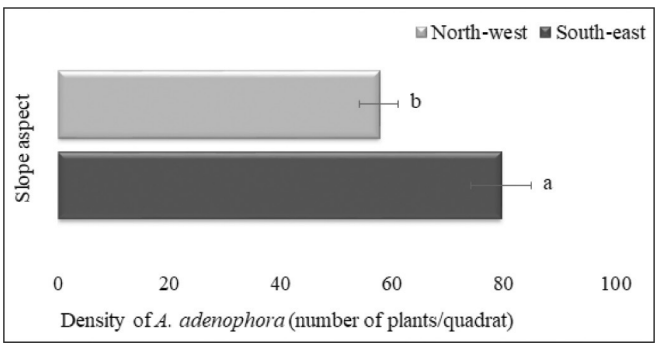

Figure 2: Densities of *A. adenophora* in south-east and north-west facing slopes in Takhtar Community Forest, Chitlang, Makwanpur, Nepal. Different letters to the right of the error bars indicate significant difference at 5% level of significance as determined by independent sample t-test.

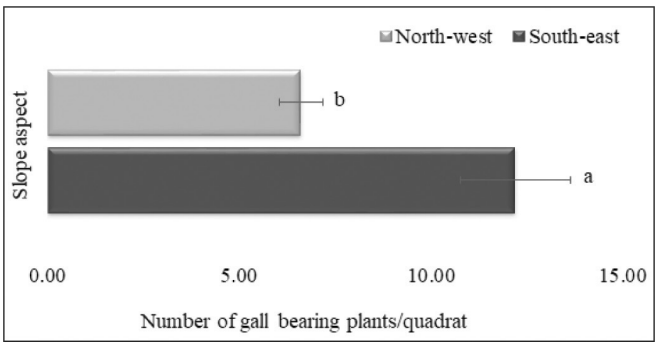

Figure 3: Number of gall-bearing plants in the south-east and north-west facing slopes of Takhtar Community Forest, Chitlang, Makwanpur, Bagmati Province, Nepal. Different letters to the right of the error bars indicate significant difference at 5% level of significance as determined by independent sample t-test.

Table 1: Percent of gall-bearing plants/quadrat in Takhtar Community Forest, Chitlang, Makwanpur, Bagmati Province, Nepal

S.N.	No. of Plant/quadrat	Total gall plant/quadrat	% of gall plants/quadrat
South-east	79.45±5.35	12.2±1.45	15.36±1.64
North-west	57.55±3.54	6.6±0.57	11.47±1.62

Similarly, the number of galls formed by *P. utilis* per quadrat (gall density) also differed by the slope aspects. The number of galls per quadrat was the highest in the south-east facing slope that was 14.35±1.7 while there were only 7.9±0.73 galls/quadrat in the north-west facing slope (U = 79.5, p = 0.001, Figure. 4a). However, the numbers of galls per plant per quadrat in the two slope aspects did not show any significant difference. The number of galls per plant in the south-east and north-west facing slopes were 0.19±0.021 and 0.15±0.021 galls/plant/quadrat, respectively (Figure 4b, t = 1.28, df =38, p = 0.214).

The results of our study showed that the density of *A. adenophora* (erect stems from rootstocks) differs with the slope aspect. The density was high in the south-east facing slope than the north-west facing slope of the studied forest (Figure 2). Similarly, the densities of gall bearing plants (number of gall bearing erect stems/quadrat) and galls (number of galls/quadrat) were also affected by the slope aspect. Both of these parameters were high in the south-east facing slope (Figures 3 and 4) but, interestingly, the number of galls per plant per quadrat was similar in both types of slope aspects (Figure 4b).

As the *A. adenophora* is one of the aggressively invading alien species in all types of habitats such as roadside, fallow lands, and forests in different physiographic zones (Tiwari et al., 2005; Thapa et al., 2016; Poudel et al., 2019), it is interesting and essential to observe various factors affecting its distribution and abundance. Such studies would have great significance in understanding the invasion behavior of *A. adenophora* and the information could be useful for developing strategies for its control and management. In the context of having limited information regarding the effects of topographic factors on *A. adenophora,* this study has revealed that, in the area under study, the south-east slope may be more suitable for luxuriant growth of *A. adenophora* in comparison to the north-west slope.

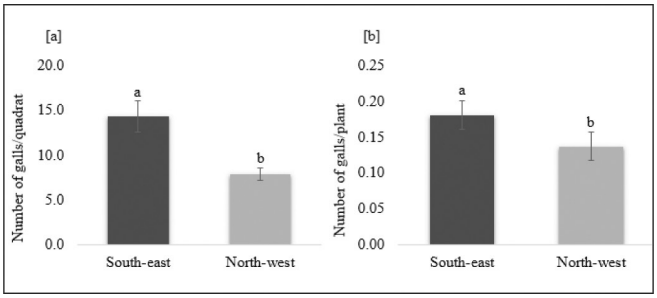

Figure 4: Numbers of galls per quadrat (gall density) [a] and numbers of galls per plant per quadrat [b] in the south-east and north-west facing aspects of Takhtar Community Forest, Chitlang, Makwanpur, Bagmati Province, Nepal. Different letters above the error bars indicate significant difference at 5% level of significance as determined by Mann-Whitney U-test in Figure 4a and independent sample t-test in Figure 4b.

Chaudhary et al. (2019) have shown higher concentration of *A. adenophora* in the north and north-west direction and lower concentration in the south-east direction in Gokerneshwergad watershed of Kailash Sacred Landscape (KSL) in western Himalaya, India. This finding is contrasting to the findings of our study. Based on these findings, it can be expected that there might be other associated factors that interact with the slope aspect to influence the invasion severity of *A. adenophora.*

Previous studies have confirmed that the density and cover of *A. adenophora* are high in the open canopies (Song et al., 2017; Thapa et al., 2016). Light is another factor affecting *A. adenophora.* Wang et al. (2004) have concluded that *A. adenophora* has ability to grow healthily in a wide range of light intensities and Zheng et al. (2012) concluded that the plant's performance can be decreased with low irradiance. Regarding the soil factor, some studies have focused on the effect of *A. adenophora* on soil physicochemical and biological parameters (Thapa et al., 2017; Zhao et al., 2019; Wu et al., 2020) but, during the invasion, what type of soil is preferred by *A. adenophora* is rarely explored. Wang and Feng (2005) have concluded that the *A. adenophora* invasion can be promoted by enhanced soil nitrogen because it is able to acclimate to a wide range of nitrogen environments and grow better in

higher nitrogen environments. Therefore, extensive studies to correlate soil physicochemical parameters with *A. adenophora* density and abundance are recommended.

Another objective of this study was to know whether there is a difference in the number of galls formed by *P. utilis* between the south-east and north-west slopes as the information regarding the effect of slope aspect on the galls was deficient. A study conducted by Poudel et al. (2020) showed that elevation is an important abiotic factor having a strong effect on gall abundance and gall size. They found high abundance and the size of gall abundance at mid-elevation (1940 to 2000 m asl). Our study has revealed that, in the study area, the insect prefers the south-east facing slope for breeding activities because the gall bearing plants and the gall density were high in this aspect comparing to the north-west aspect.

It can be assumed that the high density of galls might be due to the high density of *A. adenophora* on the south-east aspect but insect breeding may not only depend on the plant density. Table 1 shows that the percent of gall bearing plants per quadrat in the study sites. This percent can also be considered as the infestation rate of the gall fly. As the infestation rate in the south-east facing slope is higher than the north-west facing slope it can be anticipated that the slope aspect is an important abiotic factor affecting the galls of this insect in *A. adenophora*.

The number of galls per plant per quadrat was not significantly different in the two slope aspects. It might be due to less number of branches in the individual plants in the study sites which was seen during the study in the field. Li et al. (2006) studied in the western Panzhihua Prefecture, Sichuan Province, Southwest China and found that the parasitism rate of *P. utilis* is high in the humid habitat and the parasitization rate of branches differs with the age of plants i.e., the seedlings and one-year-old plants are highly infected by the insect than the older plants. Hence, it can be expected that the insect parasitization may depend on geography, local environmental conditions, vegetation of the area and invasion age (time of invasion and age of plants). Therefore, future studies should be conducted considering these factors to understand the effectiveness of *P. utilis* as a biological control agent of *A. adenophora* in different geographical locations.

In conclusion, the slope aspect influences the density of *A. adenophora,* galls formed by *P. utilis* and gall density. It indicates that the slope aspect is one of the abiotic factors affecting the parasitization by *P. utilis* on *A. adenophora.* As the explorations on the effect of abiotic and biotic factors on the parasitization of *P. utilis* in the *A. adenophora* invaded regions are very deficient, future studies should be focused on these factors to understand the effectiveness of *P. utilis* as a biological control agent of *A. adenophora*.

Acknowledgements

The authors would like to acknowledge Prof. Dr Ram Kailash Prasad Yadav, Head of Department, Central Department of Botany, Tribhuvan University, Kathmandu, Nepal and Prof. Emeritus Dr Pramod Kumar Jha for providing all kinds of support. University Grants Commission Nepal is acknowledged for providing research grants for the study.

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