

Research article

Germination response of herbaceous plant species to aqueous extracts of invasive alien weed *Parthenium hysterophorus*

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Abstract

Parthenium hysterophorus is a highly invasive weed in tropical and subtropical regions of the introduced range, and one of the mechanisms suggested to explain the invasion success is allelopathic interference with co-occurring species in invaded habitats. In this study, we examined impacts of aqueous extracts (5%, w/v) of leaf and inflorescence of *P. hysterophorus* on seed germination of 19 herbaceous plant species of Nepal, representing both native as well as alien species. Seeds of test species were collected from roadside vegetation and fallowlands of four urban areas in central Nepal where *P. hysterophorus* is invading. Germination response of test species to aqueous extracts of *P. hysterophorus* varied significantly across the species; it ranged from complete suppression (*Dactyloctenium aegyptium*) to slight enhancement (*Senna occidentalis*). Out of 19 test species, four were sensitive, three moderately sensitive, and ten resistant with relative germination <33%, 33–66% and >66% in extracts, respectively. Leaf extract of *P. hysterophorus* has slightly higher inhibitory effect (low germination of test species) than inflorescence extract. Native species appeared to be more sensitive to germination suppression by extracts than aliens. Our results suggest that allelopathic interference of *P. hysterophorus* with native species can be one of the mechanisms for invasion success of this weed in the introduced range.

Key-words: Allelopathy, Nepal, resistant species, sensitive species, suppressive plants.

Introduction

Invasive alien species are recognised as a leading global threat to the native biodiversity and ecosystems as well as to the economic activities (Pimentel *et al.* 2005; Simberloff *et al.* 2013). The success of invasive species is affected by several biological attributes of the species and the characteristics of the habitat being invaded. Allelopathy, a chemical interaction between plants, is one of the several attributes of invasive alien plants allowing them to invade and establish in a new ecosystems (Hierro and Callaway 2003). Allelopathy has been suggested as a mechanism driving alien plant

Invasion, thus forming virtual monocultures as a result of which invaders become more abundant and competitively dominant in regions of the world to which they are introduced (Hierro and Callaway 2003; Inderjit *et al.* 2008).

Parthenium hysterophorus L. (Fam. Asteraceae), a native plant of Central America, the West Indies and South America, is turned out to be an aggressive invasive weed in more than 30 countries of tropical and subtropical regions in the introduced range (Asia, Africa, Australia and Oceania) with serious impact on environment and economy (Adkins and Shabbir 2014). The weed is already wide spread in South Asia (Dhileepan and Wilmot Senaratne 2009). In Nepal, *P. hysterophorus* was first noticed in 1967 (Tiwari *et al.* 2005), but the most significant expansion of its population occurred after 1990 (Shrestha *et al.* 2015).

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Currently, it is widespread in southern plain (i.e., Tarai) and hilly areas (i.e. Siwalik and Mid Hills), while it is present only at a few locations in Mid Mountains and absent in High Mountain regions (Shrestha *et al.* 2019). For such widespread occurrence of *P. hysterophorus*, a number of strategies have been suggested to account for its invasiveness, including allelopathic potential, prolific seed production and stress tolerance among others (Bajwa *et al.* 2016).

The allelopathic potential of *P. hysterophorus* has been attributed mainly to the presence of sesquiterpene lactone, parthenin, and some phenolic acids (Kanchan and Jayachandra 1980; Belz *et al.* 2007). These allelochemicals concentrate in leaves and inflorescence, but they are also present in other plant parts (Kanchan and Jayachandra 1980). The chemical analyses, bioassay, pot culture and field studies have revealed that almost all the plant parts, including trichomes and pollens are allelopathic (Evans 1997). These chemicals inhibit the germination and growth of a wide variety of plants, including pasture grasses, cereals, vegetables, and other plant species (Mersie and Singh 1987; Adkins and Sowerby 1996; Navie *et al.* 1996), displacing native plant species and transforming grasslands, open woodlands, river banks and floodplains to monocultural *P. hysterophorus* (Chippendale and Panetta 1994; Timsina *et al.* 2011). More than a dozen of crop species have been reported to be sensitive to negative allelopathic effects of *P. hysterophorus* (Qasem and Foy 2001; Tefera 2002; Singh *et al.* 2005). But sensitivity of a large number of plant species co-occurring with *P. hysterophorus* in modified and natural ecosystems has not been examined adequately. Germination of species, like *Ageratum coyzoides* L. (Singh *et al.* 2002), *Polygonum convolvulus* L., *Urochloa panicoides* Beauv., *Cenchrus liliaris* L. (Adkins and Sowerby 1996), *Salvinia molesta* Mitchell (Pandey 1994), and *Eichhornia crassipes* (Mart.) Solms (Pandey *et al.* 1993) has been reported to be affected negatively by *P. hysterophorus*. Furthermore, the sensitivity of Nepalese wild flora against allelopathic

effect of *P. hysterophorus* has not been evaluated in large scales (but see Maharjan *et al.* 2007). As a first attempt to screen Nepalese flora against allelopathic effect of *P. hysterophorus*, we examined germination response of some native and naturalized plant species growing along with the weed to aqueous extracts of leaves and inflorescence of *P. hysterophorus*.

Materials and Methods

SEED COLLECTION

Seeds of test plant species were collected from grassland, agriculture fallow land, and roadside vegetation of four urban areas in central Nepal: Butwal (Rupandehi district), Bharatpur (Chitwan district), Hetaunda (Makawanpur district) and Kirtipur (Kathmandu district) (Figure 1, Table 1). These sites were used for livestock grazing and invaded by *Parthenium hysterophorus*. They also represent three physiographic and two climatic regions where the invasion of *P. hysterophorus* is relatively high (Shrestha *et al.* 2019).

Mature seeds of 42 plant species were collected during three field visits in August, September and October from the four sites. Seeds collected were cleaned, air dried in shade for one week, and then stored at 4°C until used in germination experiments.

At the time of seed collection, herbarium specimen of each plant species was also collected. They were identified by plant taxonomists at the Central Department of Botany, Tribhuvan University, Kathmandu. Distribution status (native vs. naturalized) of the test species were identified following Press *et al.* (2000) and Shrestha *et al.* (2018). Some of the naturalized species were considered as ‘invasive’ following Tiwari *et al.* (2005). Though, the ‘invasive’ species are the subset of the ‘naturalized’ species (Pysek *et al.* 2004), for simplicity we used them as distinct categories. We define ‘naturalized species’ as the alien species with self-replacing population in natural ecosystems but without

Table 1. Some features of seed collection sites.

Collection sites	Number of species	District	Physiographic region	Climate	Elevation (m asl)
Butwal	11	Rupandehi	Tarai	Tropical	150
Bharatpur	17	Chitwan	Siwalik	Tropical	210
Hetaunda	14	Makawanpur	Siwalik	Tropical	345
Kirtipur	8	Kathmandu	Mid Hill	Subtropical	1350

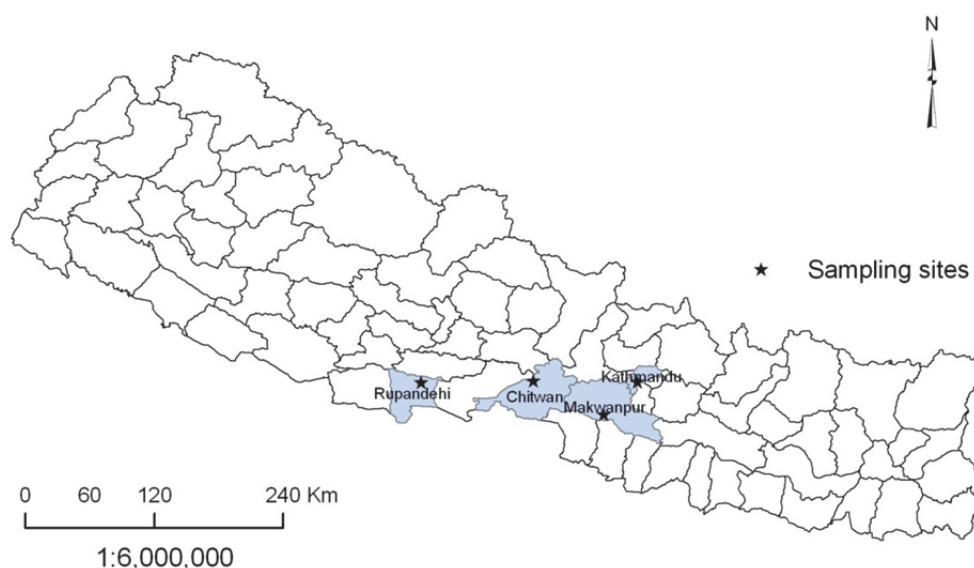


Figure 1. Map of Nepal showing locations of seed collection sites.

significant negative impacts and ‘invasive species’ to those alien species in natural ecosystems with significant negative impacts on environment and economy. Two species (*Solanum nigrum* L. and *Urena lobata* L.) were classified as ‘cryptogenic’ (Carlton 1996; Essl *et al.* 2018) since native range of these species is doubtful (<http://www.plantsoftheworldonline.org>).

GERMINATION EXPERIMENTS

Collection and processing of Parthenium hysterophorus

Aerial parts of *Parthenium hysterophorus* in full bloom were collected from Kirtipur, Kathmandu (elevation: 1350 m asl). Leaves and inflorescences were separated and shade dried for two weeks in laboratory. The dried plant materials were ground to coarse powder using blender and then stored in air-tight plastic bags at 4°C.

Extraction

Based on our previous experiences and published references, we decided to use 5% aqueous extract (w/v) of *P. hysterophorus* as test solution for seed germination experiments. It has been shown that the sensitive species showed significant suppression in germination and growth at concentration $\leq 5\%$ extract (w/v) of plant parts of *P. hysterophorus* (e.g., Mersie and Singh 1987; Pandey *et al.* 1993; Pandey 1994; Tefera 2002; Maharjan *et al.* 2007). Fifty grams powdered leaves and inflorescence were taken separately in conical flask containing 1000 mL distilled water and shaken for about five minutes. The conical flasks were then closed by

aluminum foil and maintained at cool ($<10^{\circ}\text{C}$) and dark place for 24 hours. The mixture was again shaken and filtered through double layer of muslin cloth; the residue was pressed between palms to squeeze out any amount of extract left with the residue. The extract was successively filtered through normal and then Whatmann no 1 filter paper to get clear solution. At each time, the residue was washed with a small amount of distilled water to ensure complete removal of soluble substances. The filtrate was collected in a volumetric flask (1000 mL) and the final volume was adjusted by adding required amount of distilled water. The extract was stored at 4°C.

Treatments and observations

Clean and dry Petri dishes (diam. 9 cm) were lined with double layer of filter paper which was flooded by 10 mL extracts (leaf or inflorescence) or distilled water (control). Three hundred healthy and uniform seeds of each test species were selected, and 100 seeds were transferred to Petri dishes with plant extracts or distilled water. For small seeds, three Petri dishes were used with 33, 33 and 34 seeds in each; for large seeds five dishes were used with 20 seeds in each. In each dish, the seeds were maintained at equal distance. The Petri dishes with seeds were placed near window inside the laboratory. They received 4–5 hours of direct sun light each day. The positions of Petri dishes were changed in every two days to avoid any positional effect. During the experimental period, the ambient temperature of the room measured

at 1 to 2 PM was $19\pm3^{\circ}\text{C}$ and relative humidity was $50\pm6\%$.

Seeds placed in germination were examined once in every two days for 60 days. At each time of examination, number of seeds germinated was counted and they were removed. Emergence of radicle was considered as a criterion for germination (Baskin and Baskin 1998). Small amount of distilled water was added when needed to maintain moisture in the filter papers.

DATA ANALYSIS

Total germination at the end of experiment (60 days) was expressed in percentage. Out of 42 species, germination of 19 species was $>33\%$ in control, and thus only these species were included in further data analyses. The rate of germination was calculated as Timson's index which was obtained as Σn , where n is the cumulative germination percentage for each observation (Timson 1965; Baskin and Baskin 1998; Hierro *et al.* 2009). Higher the value of index, faster is the rate of germination. In this experiment, the total number of observations was 30 (alternate days for 60 days), and the value of Timson's index can range from 0 (no germination) to 3000 (100% germination in first observation). Based on the relative germination in leaf and inflorescence extracts of *Parthenium hysterophorus*, the test species were categorized into three sensitivity classes, i.e. sensitive ($<33\%$), moderately sensitive ($33\text{--}66\%$) and resistant ($>66\%$). The relative germination of each species was calculated as follows. For each species, mean germination in extracts (sum of germination percentage in leaf and inflorescence extract/2) was calculated. This value was then expressed as the percentage of control (mean germination in extract $\times 100/\text{germination in control}$) to obtain relative germination in extract. To understand the relative effects of leaf and inflorescence extracts on germination of test species, germination percentage of all 19 species were pooled separately for control and treatments, and mean values were compared by paired samples t-test using Statistical Package for Social Sciences (SPSS, ver. 16).

Results

For pooled data of all 19 species, the germination in leaf and inflorescence extracts were significantly lower than in control (Figure 2). Mean germination in leaf extract

was lower than in inflorescence, but the difference was marginal ($p = 0.056$, paired samples t-test).

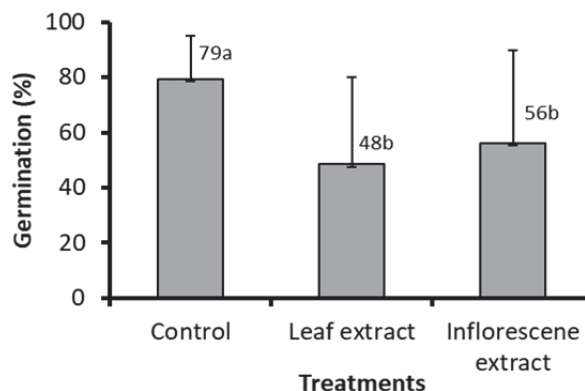


Figure 2. Mean seed germination of 19 herbaceous plant species combined in different treatments. Mean values were compared by paired samples t-test. Values followed by different alphabets were significantly different at $p=0.01$. Error bar indicates standard deviation.

Based on the relative germination in extracts, six species were sensitive with very low germination in *Parthenium hysterophorus* extracts, three species moderately sensitive, and ten species resistant (Table 2). All three species of Poaceae under test were sensitive, while test species belonging to Asteraceae (2 species) and Fabaceae (5 species) were resistant.

Seven resistant species out of ten are alien (either naturalized or invasive, Table 2) and four (*Bidens pilosa* L., *Mesosphaerum suaveolens* (L.) Kuntze, *Senna occidentalis* (L.) Link and *S. tora* (L.) Roxb.) are invasive in Nepal. Among the sensitive (6) and moderately sensitive (3) species, three (33%) are alien and one of them (*Amaranthus spinosus* L.) is invasive.

Among the sensitive species, suppression of seed germination was strong among the species of Poaceae, such as *Dactyloctenium aegyptium* and *Chrysopogon aciculatus* (Figure 3, Table 3). Germination of *D. aegyptium* seeds was completely inhibited by the extracts while the relative germination of *Chrysopogon aciculatus* seeds was 15% (Appendix 1). In *Amaranthus spinosus*, *Calotropis gigantea* and *Eragrostis atrovirens*, germination of extract-treated seeds initiated in 22 to 36 days after germination in control (Figure 3). However, in *Chrysopogon aciculatus* and *Urena lobata*, seed germination initiated almost simultaneously in extracts and control, but the germination rate was very low in extracts as compared to control (Table 3).

Table 2. Species grouped into sensitivity categories based on the relative germination in aqueous extracts of leaves and inflorescence of *Parthenium hysterophorus*.

SN	Name of species (Family)	Seed collection site	Distribution status	Sensitivity category
1	<i>Amaranthus spinosus</i> L. (Amaranthaceae)	Hetaunda	Invasive	Sensitive (relative germination <33%)
2	<i>Calotropis gigantea</i> (L.) W.T. Aiton (Apocynaceae)	Butwal	Native	
3	<i>Chrysopogon aciculatus</i> (Retz.) Trin. (Poaceae)	Hetaunda	Native	
4	<i>Dactyloctenium aegyptium</i> (L.) Willd. (Poaceae)	Hetaunda	Naturalized	
5	<i>Eragrostis atrovirens</i> (Desf.) Trin. ex Steud. (Poaceae)	Kirtipur	Native	
6	<i>Urena lobata</i> L. (Malvaceae)	Kirtipur	Cryptogenic	Moderately sensitive (relative germination 33–66%)
7	<i>Cynoglossum lanceolatum</i> Forssk. (Boraginaceae)	Butwal	Native	
8	<i>Solanum aculeatissimum</i> Jacq. (Solanaceae)	Bharatpur	Naturalized	
9	<i>Solanum nigrum</i> L. (Solanaceae)	Bharatpur	Cryptogenic	Resistant (relative germination >66%)
10	<i>Bidens pilosa</i> L. (Asteraceae)	Hetaunda	Invasive	
11	<i>Senna occidentalis</i> (L.) Link (Fabaceae)	Bharatpur	Invasive	
12	<i>Senna tora</i> (L.) Roxb. (Fabaceae)	Butwal	Invasive	
13	<i>Cirsium verutum</i> (D. Don) Spreng. (Asteraceae)	Kirtipur	Native	
14	<i>Crotalaria pallida</i> Aiton. (Fabaceae)	Hetaunda	Naturalized	
15	<i>Cuscuta reflexa</i> Roxb. (Convolvulaceae)	Kirtipur	Native	
16	<i>Desmodium</i> sp. (Fabaceae)	Hetaunda	Native	
17	<i>Mesosphaerum suaveolens</i> (L.) Kuntze (Lamiaceae)	Butwal	Invasive	
18	<i>Physalis peruviana</i> L. (Solanaceae)	Butwal	Naturalized	
19	<i>Trifolium repens</i> L. (Fabaceae)	Kirtipur	Naturalized	

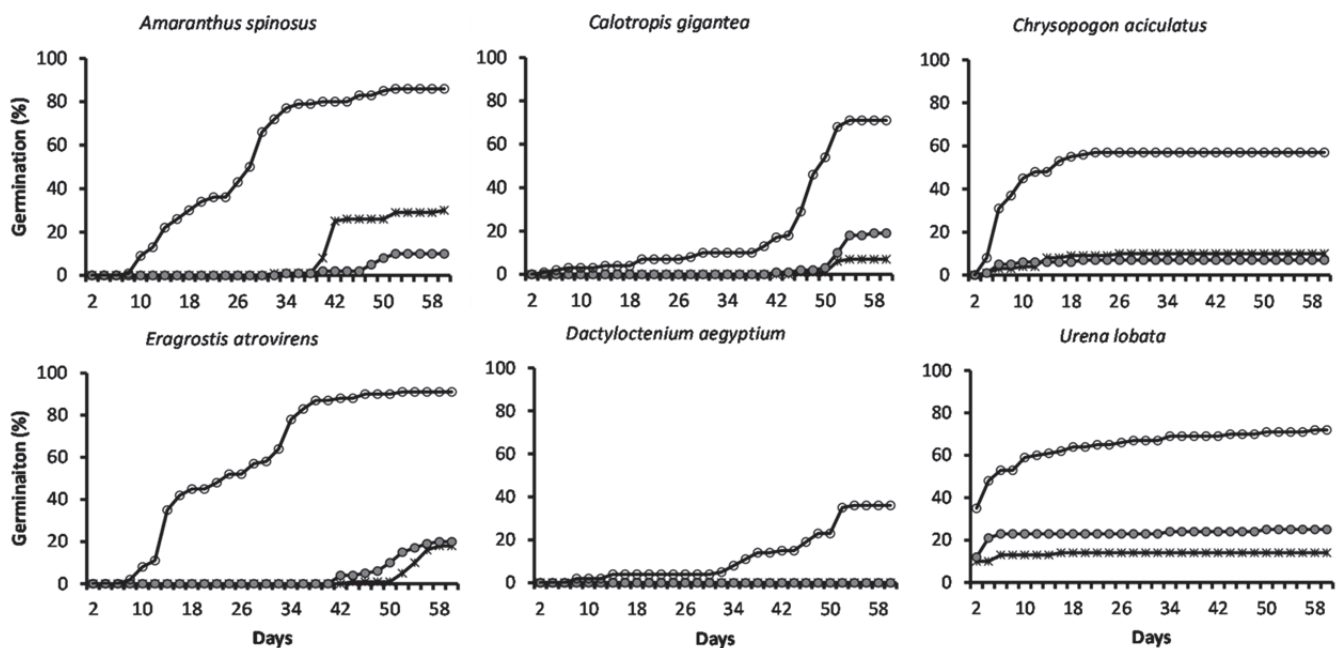
**Figure 3.** Cumulative seed germination of sensitive species over time in leaf extract (*), inflorescence extract (—●—) and control (—○—). Seeds of test species were placed in 5% leaf and inflorescence extracts of *Parthenium hysterophorus*.

Table 3. Seed germination percentage and Timson's index of the sensitive species in aqueous extracts of leaf and inflorescence (Infl.) of *Parthenium hysterophorus*. Timson's index is a measure of germination rate.

Name of species	Germination (%)			Timson's index		
	Leaf	Infl.	Control	Leaf	Infl.	Control
<i>Amaranthus spinosus</i>	30	10	86	287	74	1594
<i>Calotropis gigantea</i>	7	19	71	37	93	639
<i>Chrysopogon aciculatus</i>	10	7	57	247	188	1521
<i>Dactyloctenium aegyptium</i>	0	0	36	0	0	368
<i>Eragrostis atrovirens</i>	18	20	91	71	120	1755
<i>Urena lobata</i>	14	25	72	407	697	1939
Mean	13	14	69	175	195	1303

Among moderately sensitive species, suppression of seed germination was the highest in *Solanum nigrum* by leaf extract of *P. hysterophorus* (Figure 4, Table 4). Surprisingly, in the same species, the inflorescence extract had a slight positive effect on seed germination. However, the positive effect was not apparent when germination rate was considered (Table 4). Germination of *Cynoglossum lanceolatum* and *S. nigrum* seeds initiated almost simultaneously in extracts and control, but germination of *Solanum aculeatissimum* seeds in extracts initiated ten days after it did in control (Figure 4).

The resistant species were all dicot. Except *Cuscuta reflexa*, all resistant species had cumulative germination >70% in control and >60% in extracts (Figure 5). Among these later species, however, the germination rate of *Physalis peruviana* was very low due to delayed germination. Germination of *Senna occidentalis* was nearly equal in control and leaf extract, but it was higher by 9% in inflorescence extract as compared in control. In *Bidens pilosa*, the cumulative germination in leaf and inflorescence extracts of *P. hysterophorus* were equal but the rate of germination was lower in inflorescence extract than in leaf (Figure 5, Table 5). The germination rate was generally higher in control but in *Senna occidentalis*, *Cirsium verutum* and *Desmodium* sp. it was higher in inflorescence extract than in control.

Mean of seed germination percentage of all test species combined in leaf extract of *Parthenium hysterophorus* was slightly lower (48%) than in inflorescence (55%), but the difference was not significant (t test, $p > 0.05$). When individual species is considered, *Calotropis gigantea*, *Urena lobata* among sensitive species, *Solanum aculeatissimum* and *S. nigrum* among moderately sensitive species, and *Physalis peruviana*, *Desmodium* sp., *Mesosphaerum suaveolens* and *Senna occidentalis* among resistant species had lower seed germination in leaf extract of *P. hysterophorus*

than in inflorescence. However, germination of *Amaranthus spinosus* was higher in leaf extract than in inflorescence. The germination percentage of *Bidens pilosa* was equal in leaf and inflorescence extracts but the germination rate (Timson's index) was higher in leaf extract than in inflorescence.

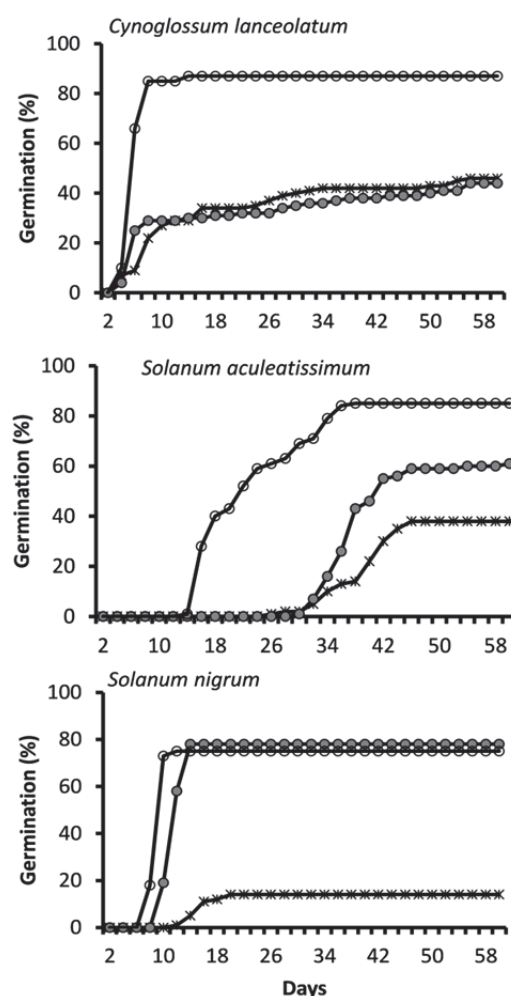
**Figure 4.** Cumulative seed germination of moderately sensitive species over time in leaf extract (*), inflorescence extract (●) and control (○). Seeds of test species were placed in 5% leaf and inflorescence extracts of *Parthenium hysterophorus*.

Table 4. Seed germination percentage and Timson's index of the moderately sensitive species in aqueous extracts of leaf and inflorescence (Infl.) of *Parthenium hysterophorus*.

Name of species	Germination (%)			Timson's index		
	Leaf	Infl.	Control	Leaf	Infl.	Control
<i>Cynoglossum lanceolatum</i>	46	44	87	1056	997	2419
<i>Solanum aculeatissimum</i>	38	61	85	438	727	1670
<i>Solanum nigrum</i>	14	78	75	323	1949	1966
Mean	33	61	82	606	1224	2018

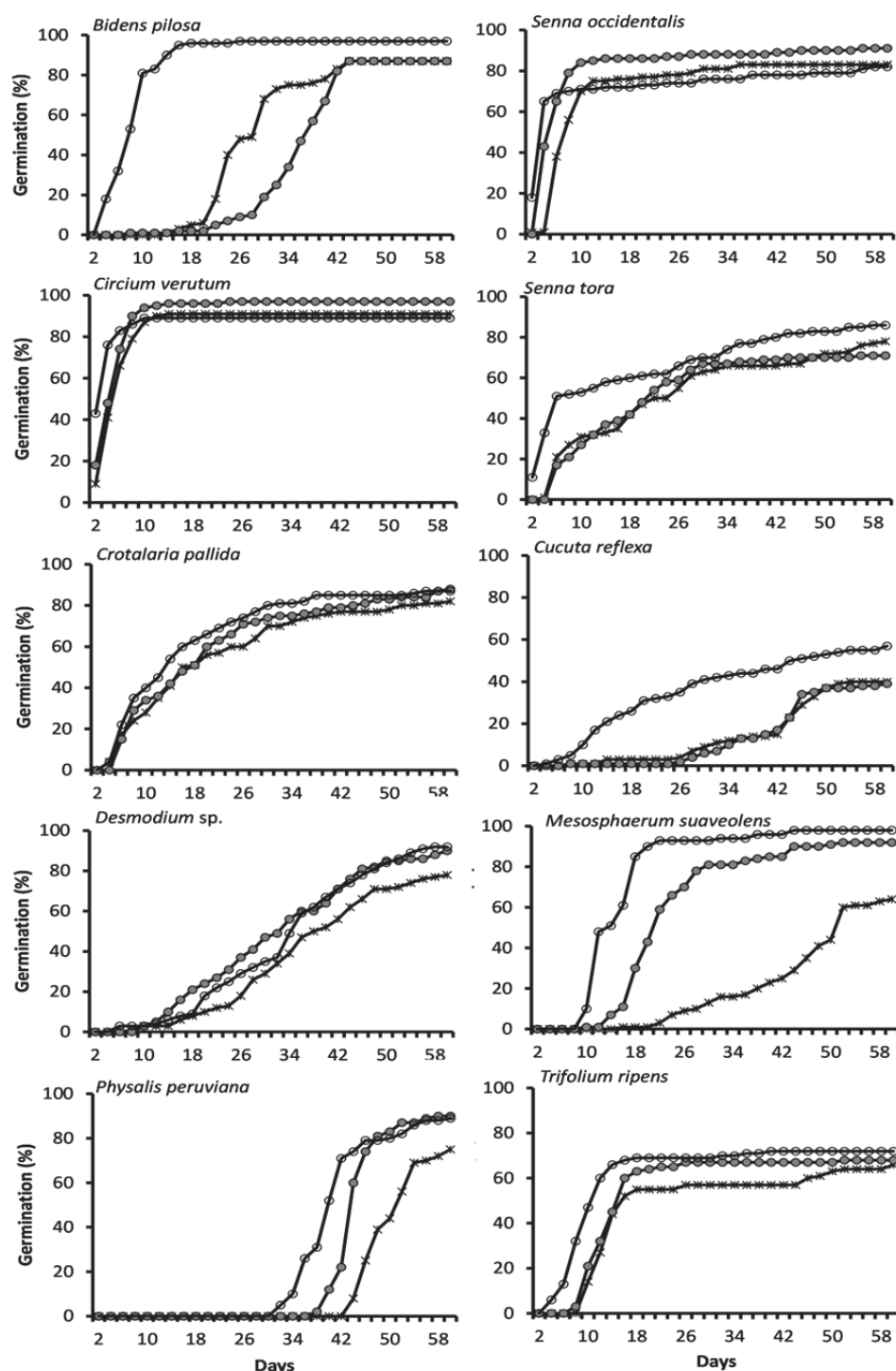
**Figure 5.** Cumulative germination of moderately sensitive species over time in leaf extract (+), inflorescence extract (x) and control (o). Seeds of test species were placed in 5% leaf and inflorescence extracts of *Parthenium hysterophorus*.

Table 5. Germination percentage and Timson's index of the sensitive species in aqueous extracts of leaf and inflorescence (Infl.) of *Parthenium hysterophorus*. Bold face indicates the highest value for the species.

Name of species	Germination (%)			Treatments		
	Leaf	Infl.	Control	Leaf	Infl.	Control
<i>Bidens pilosa</i>	87	87	97	1481	1155	2582
<i>Senna occidentalis</i>	83	91	82	2179	2477	2203
<i>Senna tora</i>	78	71	86	1594	1606	2034
<i>Cirsium verutum</i>	91	87	89	2556	2742	2602
<i>Crotalaria pallida</i>	82	88	87	1774	1876	2032
<i>Cuscuta reflexa</i>	40	39	57	441	414	1065
<i>Desmodium</i> sp.	78	90	92	1057	1381	1309
<i>Mesospaerum suaveolens</i>	64	92	98	620	1767	2262
<i>Physalis peruviana</i>	75	90	89	458	777	940
<i>Trifolium repens</i>	66	68	72	1433	1628	1849
Mean	74	80	85	1320	1550	1835

Discussion

SUPPRESSION OF GERMINATION

Germination response of seeds of the test species to leaves and inflorescence extracts of *Parthenium hysterophorus* varied widely with no germination (e.g., *Dactyloctenium aegyptium*) to >90% germination (e.g., *Mesospaerum suaveolens*, *Senna occidentalis*). This kind of species specific variation in sensitivity of seed germination and growth of test species to the allelopathic effects of *P. hysterophorus* has also been reported earlier (Mersie and Singh 1987; Adkins and Sowerby 1996; Batish *et al.* 2002; Belz *et al.* 2007; Belgeri and Adkins 2015). Seed germination of nearly half (10 out of 19) of test species, all being dicot, was not inhibited significantly (relative germination >66%) by the aqueous extracts of *P. hysterophorus*. All the three monocot species included in the present bioassay belonged to 'sensitive' category. This result is consistent with the earlier report that the parthenin, the most bioactive compound present in *P. hysterophorus*, has higher inhibitory effect on monocots than on dicots (Khosla and Sobti 1979, as cited in Navie *et al.* 1996).

Germination of *Eragrostis atrovirens* was not only suppressed but also delayed by more than four weeks. Another congeneric species *E. tef* (Zuccagni) Trotter was also found to be highly sensitive to allelopathic effects of *P. hysterophorus* (Tefera 2002). In *Senna occidentalis*, total germination as well as the rate of germination (Timson's index) was higher in inflorescence extract of *P. hysterophorus* than in control. This indicated a weak

stimulatory effect of *P. hysterophorus* on seed germination of *S. occidentalis*, a species reported to have interference effects on *P. hysterophorus* (Mahadevappa 2009).

Among the sensitive species in present categorization, *Amaranthus spinosus* has been reported to have negative interference effects on *P. hysterophorus*, thereby suppressing the growth of later species in fallow lands (Mahadevappa 2009). This contradicted the result of present laboratory experiment because the aqueous extracts of *P. hysterophorus* not only delayed the germination by about four weeks but also reduced germination from 86% (control) to 20% (extracts). There could be two reasons for this contradiction. First, it is likely that competition for resources may be more important than allelopathy for the interference between these two species in the field (e.g., San Emeterio *et al.* 2007). Second, the allelopathic interactions in the field condition may not be predictable by this kind of laboratory bioassay (Inderjit and Weston 2000).

INHIBITORY EFFECTS OF LEAF VS. INFLORESCENCE

Comparison of inhibitory effects of leaf and inflorescence extracts of *P. hysterophorus* on germination of test species showed that leaf extract had slightly higher inhibitory effect than inflorescence. In *Eragrostis tef*, leaf extract of *P. hysterophorus* had stronger inhibitory effects than flower on seed germination and seedling growth (Tefera 2002). Stronger inhibitory effects of leaves and inflorescence of *P. hysterophorus* than stem and root on growth of aquatic plants

(*Eichhornia crassipes* (Mart.) Solms and *Salvinia molesta* D.S. Mitch.) have also been reported (Pandey *et al* 1993; Pandey 1994). The inhibitory effects of *P. hysterophorus* have been mainly attributed to parthenin, a sesquiterpene lactone. The parthenin can completely inhibit germination of other weed, such as *Ageratum conyzoides* L. at 400 μ M (Singh *et al* 2002). A chromatographic estimation showed that parthenin concentration was highest in leaf (3.4% w/w, wet weight), followed by flower (1.08%), stem (0.12%) and root (<0.01%) (Subba Rao *et al.* 1976, as cited in Mahadevappa 2009). Kanchan and Jayachandra (1980) also reported higher quantity of phenolics, group of inhibitors including parthenin, in leaves than in inflorescence. With the highest concentration of parthenin and other inhibitors, leaves should have higher inhibitory effects than any other parts of *P. hysterophorus*. This theoretical prediction has been partly supported by the present results. Since the result was not consistent across the test species, the species-specific variation cannot be ruled out.

SENSITIVITY OF NATIVE VS. ALIEN SPECIES

Our results revealed that the inhibitory effect of *Parthenium hysterophorus* to other plants varied widely and was species specific. Effects of this allelopathic interference may also depend on the phytogeographic relations of the source and test plants. For example, *Centaurea maculosa* Lam., a native of Europe and invasive in North America, has been reported to suppress growth of plant species that are native of North America but has no effect to the species which are native of Europe (Thorpe *et al.* 2009). Our data also showed that majority (67%) of sensitive and moderately sensitive species (with relatively low seed germination in extracts of *P. hysterophorus*) are native of Nepal, whereas the majority (70%) of resistant species (with relatively high seed germination) were alien in Nepal but native of the Americas which is the native range of *P. hysterophorus*. Belgeri and Adkins (2015) also reported stronger negative effect by *P. hysterophorus* on growth of native species than to alien species of Australian grassland. It appears, therefore, that the inhibitory effect of *P. hysterophorus* on seed germination is stronger to native species than to alien.

IMPLICATION FOR CONTROL OF *PARTHENIUM HYSTEROPHORUS*

Nearly half of the test species were resistant to allelopathic inhibition of *Parthenium hysterophorus* for

seed germination. Among them, *Senna tora*, *S. occidentalis* and *Mesosphaerum suaveolens* have been reported to have interference effects on *P. hysterophorus*, and their promotion has been recommended for competitive replacement of *P. hysterophorus* (Mahadevappa 2009). *Senna occidentalis* can also suppress germination and growth of *P. hysterophorus* (Knox *et al.* 2011). It has been argued that the competitive species can replace *P. hysterophorus* through physical dominance and light deprivation, and allelopathy is not involved in this interference (Asha Kumari *et al.* 2010). However, Prasad *et al.* (2006) reported a reduction in seed germination and early seedling growth of *P. hysterophorus* by aqueous extracts of seeds of *Senna tora*. Another congener, *Senna uniflora* (Mill.) H.S. Irwin and Barneby (Syn. *Cassia sericea* Sw.), a native of Central America, has been reported as replacing *P. hysterophorus* through interference (allelopathic as well as competitive) in fallow land of India (Joshi 1991). All these resistant species can form dense pure stands and grow to the same, or greater, height of *P. hysterophorus*. It is likely that if they germinate earlier than *P. hysterophorus*, these species can suppress the growth of *P. hysterophorus*. However, these resistant species (*S. tora*, *S. occidentalis*, *M. suaveolens* and *Bidens pilosa*) are themselves invasive (Tiwari *et al.* 2005) and it is not wise to recommend their promotion for the control of *P. hysterophorus*. Among the three resistant species which are native to Nepal, *Desmodium* sp. is a legume and has fodder value. A number of fodder plants have been shown to suppress growth of *P. hysterophorus* in laboratory and field experiments, indicating their potential to be used as a part of integrated weed management (O'Donnell and Adkins 2005; Khan *et al.* 2013, 2014). Therefore, *Desmodium* sp. can be a potential candidate species in Nepal for use in suppressing the growth of *P. hysterophorus*. However, performance of this species needs to be evaluated under laboratory and field conditions by growing together with *P. hysterophorus*.

Conclusions

Our results suggest that allelopathic interference of *Parthenium hysterophorus* to co-occurring plant species in invaded community can be an important mechanism of the invasion process. However, the response of the associated species to allelopathic interference could be species-specific with some species being highly sensitive

while others almost not affected. Since native species appears to be more sensitive (high suppression) in germination than the alien species, the native species are likely to be suffered more than the aliens in the invaded ecosystems. Resistant species which are native and have fodder value can be a candidate to consider for suppression of *P. hysterophorus* as a part of integrated weed management. Present results have been based on the germination response of nineteen species collected randomly from four urban areas of central Nepal. Therefore, conclusions drawn from this work is only preliminary. Similar kind of screening at community level from potentially and actually invaded ecosystems can give clearer picture of sensitivity of Nepalese flora to *P. hysterophorus* invasion. This will also help to identify additional species that can be used to suppress the growth of *P. hysterophorus* in the invaded habitats.

Acknowledgements

This work was supported by grants from Nepal Academy of Science and Technology (NAST) to BBS. We are thankful to Prof. Mohan Siwakoti for plant identification and Ms. Jyoti KC for help in field and laboratory works.

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Appendix 1. Seed germination (%) of the selected species in leaf and inflorescence extracts of *Parthenium hysterophorus*. The species having seed germination >33% in control have been presented.

SN	Col No.	Scientific name	Leaf extract	Inflorescence extract	Control	Mean germination in leaf and inflorescence	Relative germination (mean germination as % of control)
1	HT04	<i>Amaranthus spinosus</i> L	30	10	86	20	23
2	HT06	<i>Chrysopogon aciculatus</i> (Retz) Trin	10	7	57	8.5	15
3	HT09	<i>Dactyloctenium aegyptium</i> (L.) Willd.	0	0	36	0	0
4	HT10	<i>Bidens pilosa</i> L.	87	87	97	87	90
5	CHT21	<i>Solanum aculeatissimum</i> Jacq.	38	61	85	49.5	58
6	BT40	<i>Cynoglossum lanceolatum</i> Forssk	46	44	87	45	52
7	BT48	<i>Calotropis gigantea</i> W.T. Aiton	7	19	71	13	18
8	BT49	<i>Physalis peruviana</i> L.	75	90	89	82.5	93
9	KRT60	<i>Eragrostis atrovirens</i> (Desf.) Trin. ex Steud.	18	20	91	19	21
10	KRT65	<i>Cuscuta reflexa</i> Roxb.	40	39	57	39.5	69
11	KRT69	<i>Cirsium verutum</i> (D.Don) Spreng.	91	87	89	89	100
12	CHT98	<i>Solanum nigrum</i> L.	14	78	75	46	61
13	HT108	<i>Desmodium</i> sp.	78	90	92	84	91
14	KRT118	<i>Urena lobata</i> L.	14	25	72	19.5	27
15	CHT135	<i>Senna occidentalis</i> (L.) Link	83	91	82	87	106
16	BT143	<i>Senna tora</i> (L.) Roxb.	78	71	86	74.5	87
17	BT153	<i>Mesosphaerum suaveolens</i> (L.) Kuntze	64	92	98	78	80
18	HT161	<i>Crotalaria pallida</i> Aiton	82	88	87	85	98
19	KRT164	<i>Trifolium repens</i> L.	66	68	72	67	93