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Prevalence of *Puccinia abrupta* var. *partheniicola* and its impact on *Parthenium hysterophorus* in Kathmandu Valley, Nepal

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Abstract

Background: *Parthenium hysterophorus* is a noxious invasive weed in tropical and subtropical regions of the world, including Nepal. Among 11 species of biological control agents released to control *P. hysterophorus* in Australia, winter rust *Puccinia abrupta* var. *partheniicola* arrived fortuitously and has established in Kathmandu Valley, Nepal, nearly a decade back. However, the prevalence and effectiveness of this rust as a biological control agent in Kathmandu remain unknown. To address this knowledge gap, a roadside survey was done at an interval of 2 ± 0.25 km in Kathmandu Valley to assess the *P. abrupta* var. *partheniicola* incidence and its impacts on *P. hysterophorus*. Infested individuals of *P. hysterophorus* were further divided into four severity classes (very low, low, medium, and high), and rust incidence was calculated. The impact of the winter rust on the growth of *P. hysterophorus* was assessed by comparing biomass and seed output of infested and non-infested individuals.

Results: Among 81 locations where *P. hysterophorus* was present in the Kathmandu Valley, winter rust infestation was observed at 98% locations. At some locations within Kathmandu Valley such as Tinkune, Kirtipur, Chabahil, Buddha Chowk, and Dhobighat, the impacts of the rust on *P. hysterophorus* were medium to high. Aboveground biomass and seed output of *P. hysterophorus* were reduced by 47% and 73%, respectively, due to winter rust infestation. The study indicates that winter rust incidence is widespread in Kathmandu Valley with very low to high levels of damages to *P. hysterophorus* depending on the localities.

Conclusion: The rust has, therefore, a potential to reduce the growth performance of *P. hysterophorus*, and it can be used as a component of integrated management of *P. hysterophorus* by introducing to other suitable areas in Nepal.

Keywords: *Parthenium* weed, Winter rust, Biocontrol, Disease incidence, Disease severity

Background

The biological control of invasive species using fungal pathogens can be a cost-effective and sustainable tool for reducing the negative impacts of invasive alien plant species in an ecosystem as these pathogens are highly host-specific (McFadyen 1998; Charudattan and Dinooor 2000; Seastedt 2015). There are several records of successful biocontrol methods using fungal pathogens in

many countries around the world, especially with rust fungi (Charudattan 2001).

Parthenium hysterophorus L. (hereafter referred to as parthenium weed) has been recognized as a major invasive weed in tropical and subtropical regions of the world since the mid-1970s with severe impacts on natural and agricultural ecosystems and human and animal health (Adkins et al. 2019). It is an annual herbaceous plant and has invaded 49 countries across the world (Adkins et al. 2019; Maharjan et al. 2020). Biological control of parthenium weed was initiated in Australia in 1976 and now has been continued to other parts of the

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world including India, Pakistan, South Africa, Ethiopia, Tanzania, and Uganda (Strathie et al. 2011; Dhileepan et al. 2019; Maharjan et al. 2020). The winter rust *Puccinia abrupta* var. *partheniicola* (H. S. Jackson) Parmelle (hereafter referred to as winter rust), a native of Argentina, Bolivia, Brazil, and Central America, is a potential biocontrol agent among 11 agents identified for the control of parthenium weed (Evans 1997; Dhileepan and McFadyen 2012; Dhileepan et al. 2019). The host range of the winter rust tested against many plant species indicated the rust to be sufficiently host-specific for the consideration as a biological control agent of parthenium weed, and the rust caused a significant decrease in vegetative growth and seed production of parthenium weed (Evans 1987; Parker et al. 1994; Fauzi 2009). With the first introduction as a potential biological control agent for parthenium weed in Australia in 1990, the winter rust has been reported from various countries including Mauritius, India, Kenya, China, Ethiopia, South Africa, Nepal, Tanzania, and Pakistan with no deliberate release (Parker et al. 1994; Dhileepan and Strathie 2009; Shrestha 2012; Iqbal et al. 2020). It forms brown powdery pustules on the

upper leaf surface, stem, and inflorescences (Parker et al. 1994) (Fig. 1). There is a report of significant damage in parthenium weed by winter rust in Ethiopia and Pakistan (Taye 2002; Iqbal et al. 2020).

Like in other parts of the world, the rapid expansion of parthenium weed in Nepal has reduced species richness and altered species composition in grassland ecosystems (Timsina et al. 2011; Shrestha et al. 2015). In Nepal, winter rust was not deliberately introduced and was reported for the first time from Kathmandu Valley (Kirtipur municipality) in 2011 as a second biological control agent for parthenium weed after *Zygogramma bicolorata* L. (Shrestha 2012). Except for the reporting of its occurrence in Nepal, no further study has been conducted to investigate the prevalence and impact of this rust on parthenium weed in Nepal. Therefore, the main objective of the present study was to document the distribution of winter rust in Kathmandu Valley, assess the severity of the infestation, and analyze its impact on parthenium weed in Kathmandu Valley. The present study could potentially contribute to a management plan for parthenium weed in Nepal.



Fig. 1 Young pustules of *Puccinia abrupta* var. *partheniicola* with visible chlorosis on the leaves of *Parthenium hysterophorus* (a), rust pustules spreading on the upper surface of the leaf (b), ruptured rust pustules with curling on the leaf (c), infestation on the stem surface (d), and habit of infected *P. hysterophorus* with deformed growth (e)

Materials and methods

Study area

The study was conducted in Kathmandu Valley (27° 31'–27° 49' N latitudes and 85° 11'–85° 34' E longitude, 1250 to 1730 masl.) along the road networks (Fig. 2) during June 2019. It represents the most urbanized area of Nepal with three major cities—Kathmandu, Bhaktapur, and Lalitpur (Adhikari et al. 2012). It has a sub-tropical climate with 19–27 °C temperature in summer and 2–20 °C temperature in winter and an annual average rainfall of 1400 mm (Pant and Dangol 2009). The major roadside vegetation in the study area included *Bidens pilosa* L., *Cynodon dactylon* (L.) Pers., *Amaranthus spinosus* L., *Alternanthera sessilis* (L.) DC., *Conyza* sp., *Galinsoga* sp., *Solanum nigrum* L., *Solanum xanthocarpum* Schard. & Wendl., *Xanthium* sp., *Kyllinga brevifolia* Rottb., *Oxalis corniculata* L., and *Fimbristylis doctotoma* (L.) Vahl.

Distribution mapping

The survey was done at an interval of 2 ± 0.25 km (using scooter speedometer) along the roadsides of Kathmandu Valley except when there was no parthenium weed

(Table S1). In the case of the absence of parthenium weed at the above interval, the survey was continued in a nearby available location. A rectangle quadrat of size 2 × 1 m² was sampled at each location to count the number of plants. In each quadrat, a total number of infested and healthy individuals of parthenium weed were counted. Infested individuals of parthenium weed were further divided into five different severity classes (Table 1, Fig. S1). The identity of winter rust on parthenium weed was identified and confirmed by symptoms and microscopic observations following the description provided by Parmelee (1967) (Fig. S2).

The infestation by winter rust into parthenium weed at various locations of Kathmandu Valley was presented in the map by interpolating rust incidence percentage value in ArcGIS using geostatistical wizard tool (French et al. 2011).

Impact assessment

Among all the locations surveyed, three sites (Kirtipur—27° 40' 43.73" N, 85° 17' 24.04" E; Dhobighat—27° 40' 8.4" N, 85° 18' 23.34" E; and Buddha Chowk—27° 40' 52.62" N, 85° 14' 40.4" E) with comparatively higher

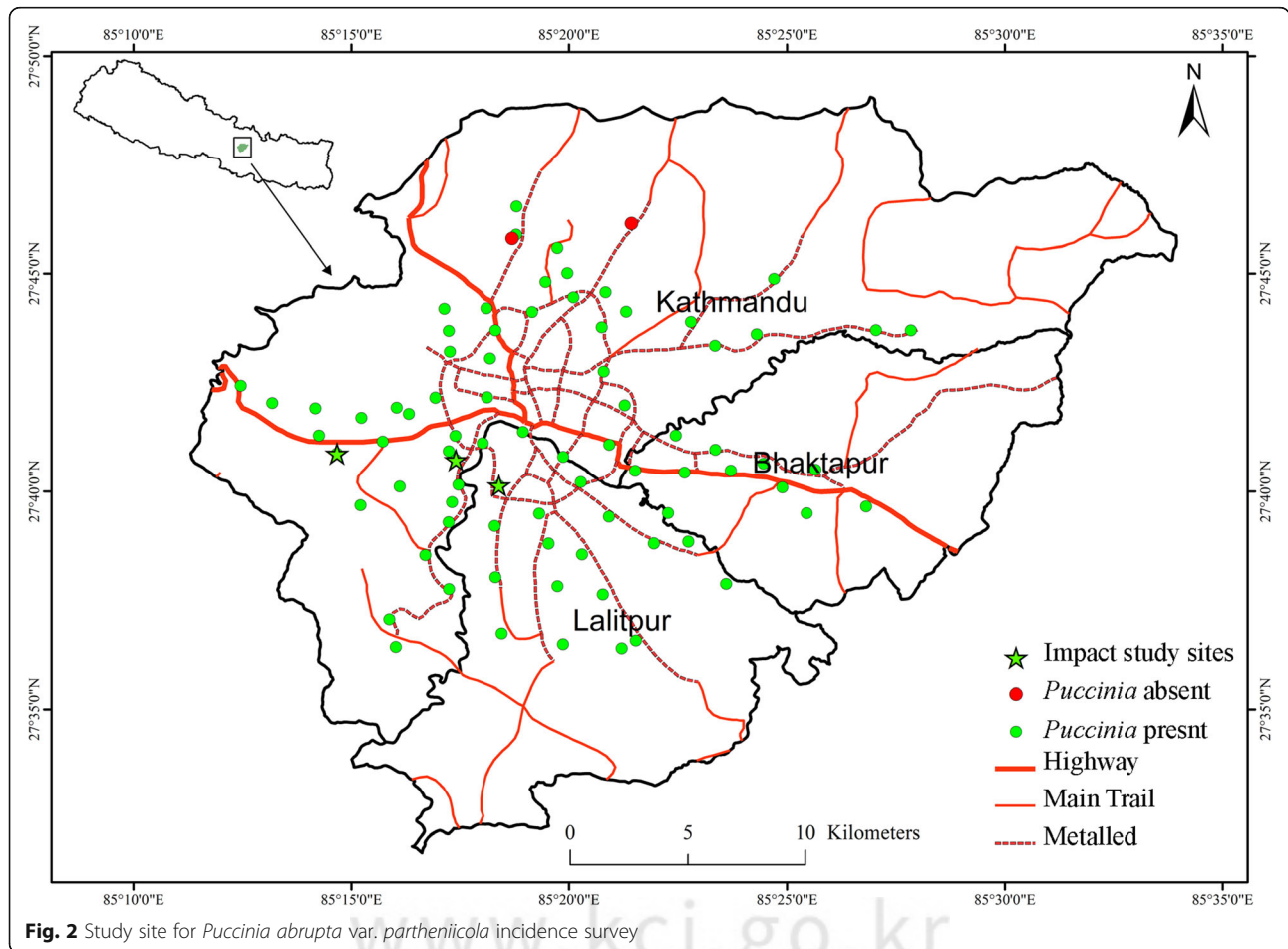


Fig. 2 Study site for *Puccinia abrupta* var. *partheniicola* incidence survey

Table 1 Categorization of infested *Parthenium hysterophorus* individuals based on the severity of infestation by winter rust

SN	Severity class	Description of severity class
1	No	Healthy plant with no infestation
2	Very low	Only a few leaves infested without any apparent impact on growth
3	Low	Premature senescence of some leaves but not apparent impact on inflorescence
4	Medium	Death of about 50% of leaves with apparent impacts on inflorescence but not senescence of plant
5	High	Death or senescence of plant

levels of infestation by winter rust were selected for impact studies (Fig. 2). At each location, three plots of 5 × 5 m² were selected for sampling. From each plot, 10 healthy individuals and 10 infested individuals (medium severity class with deformed growth) having similar basal diameters were sampled. The number of flowers present in each plant was counted. The total seed output of each individual was calculated, assuming that a single flower produces five achenes (Adkins and Shabbir 2014). The aboveground portion of each individual was packed in a paper bag and oven-dried at 70 °C for 48 h. Then, the biomass was measured by using a digital balance with an accuracy of 0.001 g.

Data analysis

Rust incidence was calculated by using the following formula (Bekeko et al. 2012):

$$\text{Rust incidence (\%)} = \frac{\text{Total number of plants infested}}{\text{Total number of plants inside the plot}} \times 100$$

The normality (Shapiro-Wilk test) and homogeneity of variance (Leven's test) for all the parameters were tested prior to choosing a parametric or non-parametric tool to analyze. The data did not meet the assumption of normality and homogeneity of variance. Hence, significant differences between variation in biomass and number of seeds among infested and non-infested individuals were tested by non-parametric Mann-Whitney—Wilcoxon test. The analyses were carried out using the R program, and all figures were also drawn in R program version 3.6.1 (R Core Team 2019).

Results

The infected leaves of parthenium weed were noted as light brown pustules on the adaxial surface (Fig. 1b). Brown pustules were even observed in the stem on severely infected plants affecting the growth of the plant. Chlorosis and curling of the leaves were also distinctly visible in highly infested conditions (Fig. 1a, c).

A total of 136 locations were surveyed inside Kathmandu Valley, of which parthenium weed was present in 81 (60%) locations. Winter rust infestation was observed in all of the locations (98% locations out of 81) where parthenium weed was present except at Phutung and Budhanilkantha (Fig. 2). The infestation of

winter rust on parthenium weed individuals was seen in most of the locations, but the severity of infestation was very low with few leaves infested and no visible impact on the growth of parthenium weed (Fig. 3). In contrast, at some locations (Tinkune, Kirtipur, Chabahil, Buddha Chowk, Dhobighat, Airport), the impact on most of the individuals of parthenium weed was very visible with severity of infestation medium to high, affecting the growth of the plant. The interpolation of the rust incidence value of various locations into the map indicated that the rust incidence was higher in the central core area of Kathmandu Valley (Fig. 4).

There was a significant impact of winter rust infestation on the aboveground biomass and total seed output of parthenium weed (Fig. 5a, b). The infestation reduced biomass and seed output by 47% and 73%, respectively.

Discussion

The absence of winter rust infestation at only two locations out of 81 surveyed locations indicates that the rust is widespread and still tending to spread in Kathmandu Valley. In Nepal, the rust was first reported from Kirtipur in 2011 during which the impact of rust on the plant was marginal (Shrestha 2012). Since then, the incidence of the rust at some locations around Kirtipur has been observed with very minimal damage to parthenium weed (Shrestha et al. 2015). Higher rust incidence in the central core area of Kathmandu Valley might be because of the first establishment of the rust in Kirtipur, and it is still tending to spread in the valley.

Low temperature and moisture present on the surface of the leaves of the host plant is the major factor determining the existence of winter rust while higher temperature reduces the extent of infection on parthenium weed (Parker et al. 1994; Taye 2002). And, in more humid, lowland regions, winter rust infestation does not affect the growth of parthenium weed (Evans 1987). There is a report of a low to high level of rust incidence depending upon the site conditions and season in the experimental sites of South Africa, too (Strathie et al. 2016). The higher rust incidence in the central core region of Kathmandu Valley during mid-July is an indication of widespread of the rust from the initial reported place, i.e., Kirtipur, and the rust might have received favorable environmental conditions for its growth.

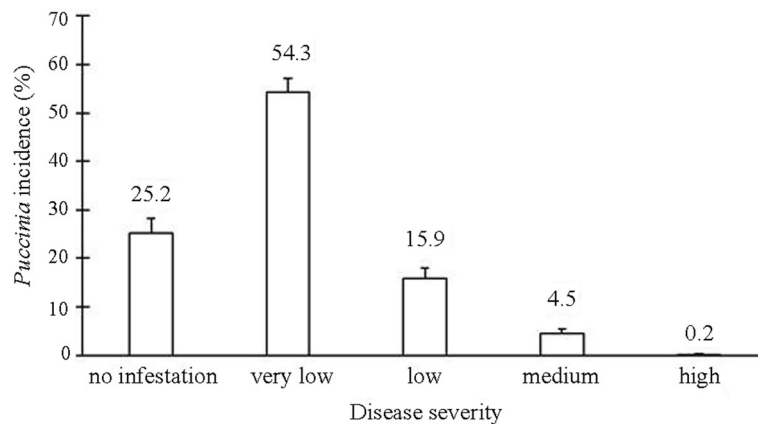


Fig. 3 Degree of infestation in *Parthenium hysterophorus* individuals by *Puccinia abrupta* var. *partheniicola* in Kathmandu Valley

Winter rust infestation includes premature senescence of the leaves to senesce, reducing the life expectancy and flower production up to 40% and 90%, respectively (Parker et al. 1994). Taye et al. (2002) reported the reduction in the seed production in parthenium weed by 42% due to the rust in Ethiopia. The present study showed the average reduction in the seed production by 73%. Winter rust infestation accelerates the senescence of the leaves of parthenium weed and reduces the amount of photosynthate products which lead to reduce the biomass, the length of flowering shoots, and the number of flowers (Parker et al. 1994). The production of a lesser number of flowers ultimately reduces seed production and restricts

the spread of the weed to some extent. In addition, infested plants would be susceptible to stress and become less competitive to the other associated plants (Parker et al. 1994).

Within 8 years since its first report, the rust has shown its widespread distribution in Kathmandu Valley with a significant visible impact on parthenium weed under a natural environment. There is also a report of winter rust outside Kathmandu Valley from Trishuli and Gorkha districts of Nepal (pers. Obs. of BB Shrestha in April to May 2019), indicating favorable environmental conditions for the growth of the rust in other parts of Nepal, too. However, the level of damage to the weed by the rust has to be studied further in those areas.

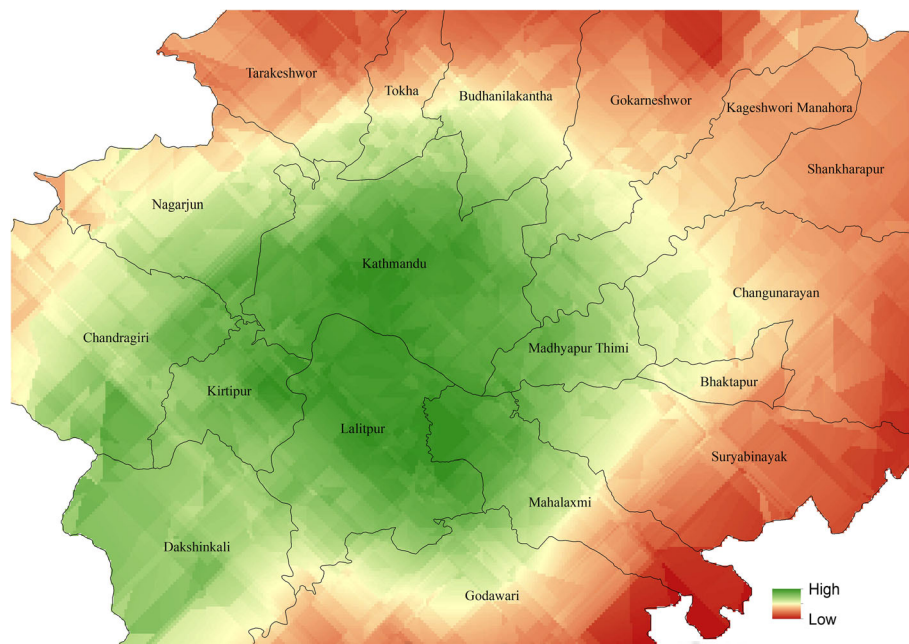


Fig. 4 Map showing *Puccinia abrupta* var. *partheniicola* incidence in various locations of Kathmandu Valley

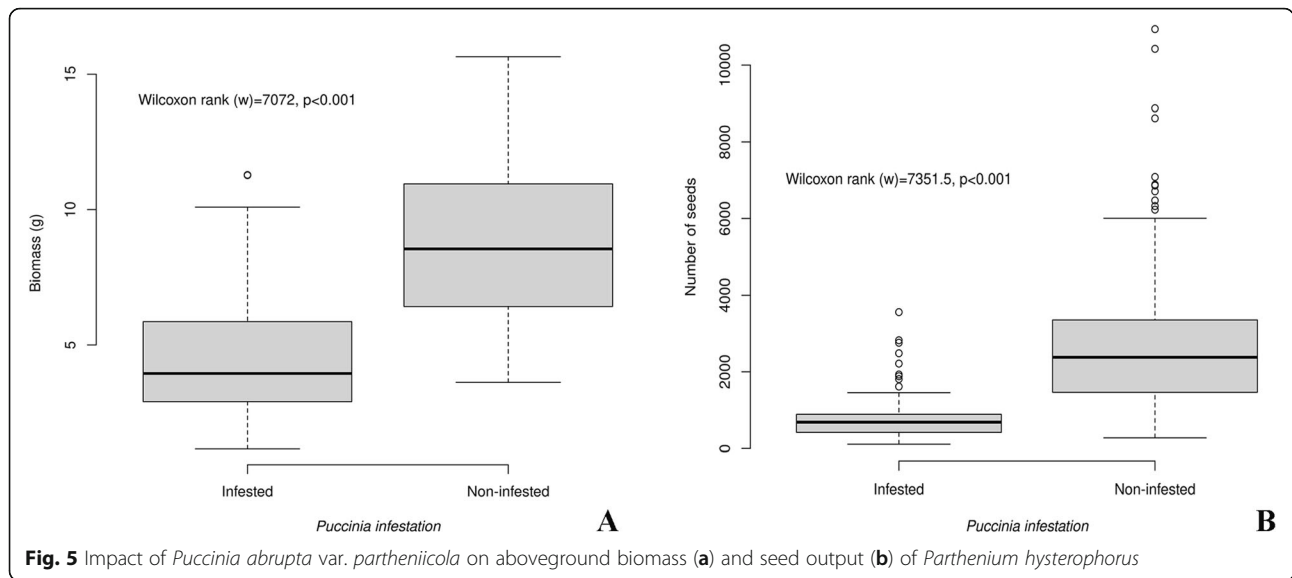


Fig. 5 Impact of *Puccinia abrupta* var. *partheniicola* on aboveground biomass (a) and seed output (b) of *Parthenium hysterophorus*

Winter rust is an autoecious, macrocyclic rust that completes its life cycle only on parthenium weed and grows well in cool, mid-altitude (1500–2500 m) regions where the rainfall varies from 700 to 1400 mm with frequent frost and night temperatures are close to the optimum temperature (below 20 °C) (Parker et al. 1994; Taye 2002). During April to May, the night temperature of Kathmandu Valley is between 12 and 15 °C, and it is close to the optimal temperature required for the proliferation of spores of winter rust and with the start of the rainy season, i.e., at the end of June during which the minimum temperature is above 18 °C, the rust gradually starts to disappear from the plant (Shrestha 2012; DHM 2016). The growth and seed production of parthenium weed inside Kathmandu Valley peaks during the rainy months, i.e., August to September (Pokhrel 2013). Hence, the disappearance of winter rust during the weed’s peak growing season could only have minimal impact on the growth of parthenium weed. However, the rust can weaken the plant and reduce seed production before the rainy season (Parker et al. 1994). Thus, it can be effective as a complementary biological control agent for parthenium weed during winter and early summer when another promising biological control agent, *Zygomorpha bicolorata*, remains quiescent in countries like Nepal, where the weed is seen at various stages throughout the year.

As parthenium weed reproduces by seed, reduced seed production likely reduces the spread of the weed and reduces competitiveness to the crop and other native plant species (Taye 2002). One of the advantages of winter rust infestation to plants is that it has the capacity to infest all green parts of plants with rapid multiplication within 8 days (Evans 1987; Parker et al. 1994). Though the rust does not kill the host completely, the present

findings as well as studies by Fauzi (2009) have also shown that it can significantly reduce the biomass and seed production of parthenium weed. So, it would appear to be a promising biological control agent in mid-altitude (> 1000 m) regions of Nepal. In addition, the introduction of winter rust to other climatically suitable parts of Nepal where parthenium weed is highly problematic could be done to control the weed to some extent.

Conclusion

The results showed that *Puccinia abrupta* var. *partheniicola* has spread across Kathmandu Valley with a significant reduction in biomass and seed production of *Parthenium hysterophorus* at some localities. It can partially control *P. hysterophorus* during winter and early summer when another biological control agent, *Zygomorpha bicolorata*, remains inactive. The identification of climatically suitable regions outside Kathmandu Valley and subsequent release of this rust in such areas can be a part of the integrated management of parthenium weed in Nepal.

Supplementary information

Supplementary information accompanies this paper at <https://doi.org/10.1186/s41610-020-00168-5>.

Additional file 1: Table S1. Total Survey sites showing total number of plants infested by *Puccinia abrupta* var. *partheniicola* and rust incidence in different locations of Kathmandu Valley. **Fig. S1.** Photographs of *Parthenium hysterophorus* infested by *Puccinia abrupta* var. *partheniicola* in different severity classes (A - healthy plant with no infestation; B - only a few leaves infested without any apparent impact on growth; C - premature senescence of some leaves but not apparent impact on inflorescence; D - death of about 50% leaves with apparent impacts on inflorescence but not senescence of plant; E - death or senescence of plant) (A, D: S. Maharjan; B, C, E: BB Shrestha). **Fig. S2.** Microscopic image of urediniospores of *Puccinia abrupta* var. *partheniicola* stained in cotton blue

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Authors' contributions

BBS and PKJ conceptualized and designed the research. AD and PKJ supervised the research. CBB guided the data analysis. SM collected and analyzed the data and wrote the manuscript. AD, BBS, CBB, MR, and PKJ critically commented and approved the final version of the manuscript. The authors read and approved the final version of the manuscript.

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Availability of data and materials

All data involved in this study are available from the corresponding authors upon request.

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Competing interests

The authors declare that they have no competing interests.

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