





## Multiple comparisons of diversity indices invaded by *Lantana camara*

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(With 5 figures)

### Abstract

Current study assessed the impact of *Lantana camara* invasion on native plant diversity in Pothohar region of Pakistan. The approach used for study was random samplings and comparisons of diversity indices [number of species (S), abundance (N), species richness (R), evenness (J'), Shannon diversity index (H') and Simpson index of dominance ( $\lambda$ )] with two categorical factors i.e., invaded and non-invaded (control). Control plots harboured by an average of 1.74 more species/10m<sup>2</sup>. The control category was diverse (H'=2.56) than invaded category (H'=1.56). The higher value of species richness in control plots shows heterogeneous nature of communities and *vice versa* in invaded plots. At multivariate scale, ordination (nMDS) and ANOSIM showed significant magnitude of differences between invaded and control plots at all sites. The decrease in studied diversity indices in invaded over control sites indicated that plant communities become less productive due to *Lantana* invasion.

**Keywords:** phytosociological study, plot comparisons, multivariate analysis, non-metric multidimensional scaling, invasion impacts, diversity conservation.

### Análises múltiplas de índices de diversidade da invasiva *Lantana camara*

#### Resumo

O presente estudo avaliou o impacto da invasão de *Lantana camara* na diversidade de plantas nativas na região de Pothohar, no Paquistão. A abordagem utilizada para o estudo foram a amostragem aleatória e a comparação de índices de diversidade, como número de espécies (S), abundância (N), riqueza de espécies (R), equitabilidade (J'), índice de diversidade de Shannon (H') e índice de dominância de Simpson ( $\lambda$ ), com dois fatores categóricos, ou seja, invadidos e não invadidos (controle). As parcelas não invadidas tinham, em média, 1,74 espécie a mais / 10 m<sup>2</sup> que parcelas invadidas. A categoria controle foi mais diversa (H' = 2,56) do que a categoria invadida (H' = 1,56). O maior valor da riqueza de espécies em parcelas de controle mostra a natureza heterogênea das comunidades, e vice-versa, em parcelas invadidas. Na escala multivariada, ordenação (nMDS) e ANOSIM mostraram magnitude significativa das diferenças entre as parcelas invadidas e controle em todos os locais. A diminuição nos índices de diversidade estudados em locais invadidos por controle indicou que as comunidades de plantas se tornam menos produtivas por causa da invasão de *Lantana*.

**Palavras-chave:** estudo fitossociológico, comparações de parcelas, análise multivariada, escala multidimensional não métrica, impactos da invasão, conservação da diversidade.

#### 1. Introduction

Global Invasive Species Program (GISP, 2020) defines an invasive species as “[...] non-native organisms that cause/have the potential to cause harm to the environment, economies, or human health”. Unwanted effects of such species may include reduced soil value, an increase in the

rate of soil erosion, water bodies choking thus affecting the quality of water, alteration in the composition of natural fauna and flora, risk to human health and economic losses (Marwat et al., 2010), soil nutrient composition, hydrology modification, forest fire cycle and other ecosystem processes

(Dogra et al., 2010), reduction in water availabilities, agricultural yields and grazing areas, contributing to spread of vector borne diseases (Etana, 2013). Due to their potential to outcompete and replace native species, invasive species are the second leading cause of biodiversity loss after habitat destruction. Keeping in view impact of invasive weeds on the environment, article 8 (h) of the Convention on Biological Diversity (CBD) signed by 161 countries at the Earth Summit in 1992; urges the parties to “[...] prevent the introduction of, control, or eradicate those alien species which threaten ecosystems, habitat or species [...]” (Qureshi et al., 2014, p. 408). The impact of exotic invasive plants on native organisms is widely acknowledged but poorly understood. Studying the community level impacts of invader identify its potential effects and provide valuable information for management and nature conservation strategies (Hejda et al., 2009).

Sage plant is medium-sized, perennial, aromatic, ornamental shrub, native to the Neotropics. It is now established in over 60 countries, rated among top ten worst weeds around the world (Qureshi et al., 2014). The shrub was introduced throughout the tropics and subtropics during the late 19th century, often used as a hedge plant (Shaukat et al., 2003) (Figure 1). Adverse effects of *Lantana* on ecosystems include damaged ecosystem services, soil erosion, reduced native biodiversity, encroaching on agricultural land, animal poisoning, harboring disease vectors and allelopathic effects resulting in reduced or no growth of associated flora. Phenotypic plasticity, high reproductive potential, immunization to grazing pressure, allelopathy and fire tolerance contributes to its invasiveness (Bhakat and Maiti, 2012). *Lantana* is strongly allelopathic and interferes with the growth and development of a wide range of plants, including ferns, vines, crops and other plants even its own populations (Ambika et al., 2003). The major allelopathic compounds found in *Lantana* weed are salicylic acid, gentisic acid,

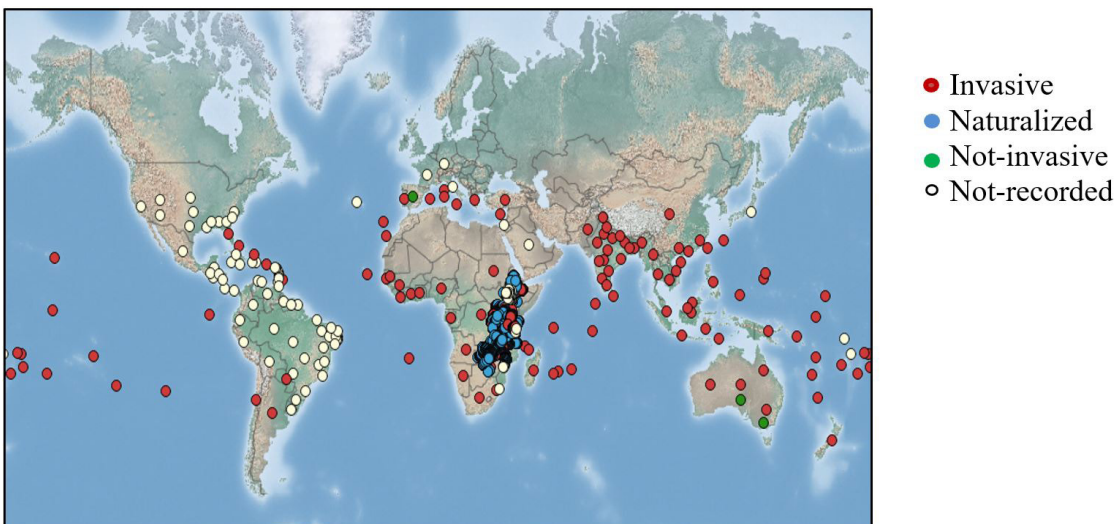
coumarin, p-hydroxybenzoic acid, ferulic acid, lantadene A, 6-methyl coumarin, lantadene B, oleanolic acid, lantalonic acid, icterogenin, lantolonic, ursolic acid and oleonic acid (Yadav et al., 2016).

*Lantana* is one of the worst weeds presently known in Pakistan. No previous study has reported from Pothohar region regarding its ecological impacts. The current study was carried out to find out (1) what is the effect of *Lantana* weed on diversity indices in different districts of the Pothohar region (assuming each district as ‘site’); (2) do the effects on diversity differ between different sites (districts) in the area?

## 2. Material and Methods

### 2.1. Study area

The Pothohar is a north-eastern plateau in Pakistan, making the northern part of Punjab. It edges Azad Kashmir (the western parts) and Khyber Pakhtunkhwa (southern parts). Pothohar Zone extends from 32.5°N to 34.0°N Latitude and 72°E to 74°E Longitude and lies between Indus and Jhelum River. The plateau expands from salt range northward to the foothills of Himalayas. The Pothohar region embraces Jhelum (32.9405°N, 73.7276°E), Islamabad (33.73°N, 73.09°E), Attock (33.76°N, 72.36°E), Rawalpindi (73.04°E, 33.59°N), and Chakwal (72.85°E, 32.93°N) districts. Total area of Pothohar region is 28488.9 Sq. Km. (Rashid and Rasul, 2011). Pothohar region has an extreme climate with hot summers and cold winters. Weather is divided into four seasons; Cold (December-March); Hot (April-June); Monsoon (July-September) and Post-Monsoon season (October-November). This area practices an average annual rainfall of 812 mm, about half of which occurs in the Monsoon months (July-September). The mean maximum temperature rises till the month of June and then falls appreciably with the advent of rains being the coldest in January (14.62-18.7°C). Average temperatures



**Figure 1.** Distribution map and invasion status of invasive *L. camara* around the globe.

range from 14°C in January to 37°C in June (Pakistan Meteorological Department, University Road Karachi, Pakistan). The region has broadly four types of soil; loess, river alluvium, residual and piedmont alluvium. Due to dynamic climate and combination of hills and plains, Pothohar region is rich in biodiversity (Shabbir et al., 2012; Ghufuran et al., 2013).

### 2.2. Experimental design

Field work was carried out during July-August (being the maximum growth period of plants), 2016. The effect of invasion was studied in each of five districts (Attock, Chakwal, Jhelum, Islamabad & Rawalpindi). Ecological indices for selected invaders were calculated and compared at various sites. The sampling technique was random. For each district six invaded and six non-invaded paired vegetation plots (each 3.16×3.16m in size, i.e., 10m<sup>2</sup> in the area) were sampled. The plots were chosen to cover a range of site conditions and vegetation types in which the invader achieves dominance in the invaded communities. Plot of invaded vegetation ('invaded plot') where the invader showed dominance was considered as 'treatment' and a second vegetation plot, usually 0.5-1 km apart from treatment, where invader has no dominance ('non-invaded plot') was considered as the "control". The estimated density of the weed in the area across locations was 4/m<sup>2</sup>. In all, 60 vegetation plots were sampled (consisting of 6 paired samples per district, and hence 30 treatments; 30 controls for the entire Pothohar region) (Figure 2). Within each randomly chosen plot (10m<sup>2</sup> in area), all vascular plant species in control and invaded plots were identified to species level.

### 2.3. Data analyses

Species frequency data were created and invasion impacts of *L. camara* on local flora were assessed by calculating and comparing ecological indices including Margalef's index of richness, Shannon-Weaver index of diversity, Simpson index of dominance and index of evenness for control and invaded sites. These parameters were calculated according to equations 1-4:

$$\text{Margalef's index of richness (R)} = \frac{S-1}{\ln N} \quad (1)$$

Where, N = Total number of individuals  
S = Total number of species.

$$\text{Shannon - Weaver index of diversity (H')} = -\sum_{i=1}^S \left( \frac{n_i}{N} \times \ln \frac{n_i}{N} \right) \quad (2)$$

Where, N = Total number of individuals of all species  
n = Actual number of individuals of one species.

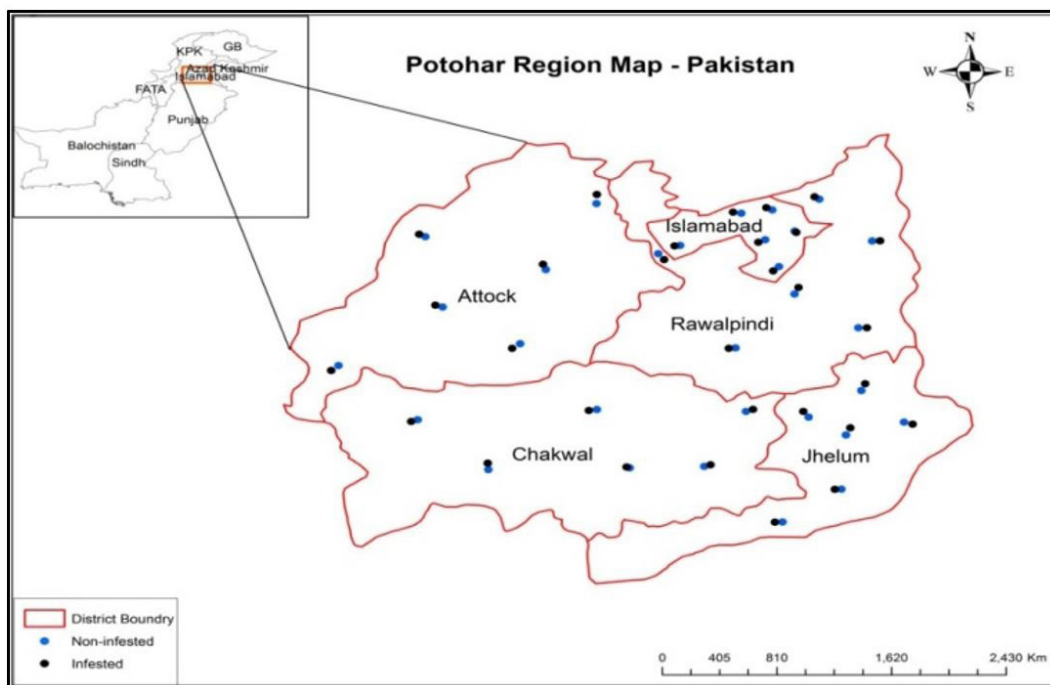
$$\text{Simpson index of dominance } (\lambda) = 1 - \frac{\sum_{i=1}^S p_i (n_i - 1)}{N(N-1)} \quad (3)$$

N = Total number of individuals of all species  
n = Number of individuals of one species

$$\text{Index of evenness (E)} = \frac{H'}{\ln S} \quad (4)$$

Where H' is Shannon's index  
S = Number of species.

Rarefaction curves were plotted to determine if sampling was adequate in each district using observed, Coleman's, Jackknife, Bootstrap and Chao2 models in



**Figure 2.** Distribution of plots for impact analysis of *L. camara* in Pothohar region.

PRIMER v. 7 (Clarke and Warwick, 2001). All gave comparable results; consequently only that of real (observed) data are presented. Data were then subjected to univariate and multivariate analyses of non-metric multidimensional scaling procedure (Clarke and Gorley, 2015). Data were log transformed to achieve criteria of normality (evenness and Simpson index of diversity). For invasion impact analysis, diversity indices including total number of species (S), abundance (N), species richness (R), species evenness (J'), Shannon index of diversity (H') and Simpson index of dominance ( $\lambda$ ) were calculated for control as well as for invaded plots. The above ecological indices were subjected to analysis of variance (ANOVA) with invasion status and districts as factors using IBM SPSS v. 21. Differences between ecological indices for five districts were individually tested for significance between invaded and control plots by multiple comparisons tests of t-test. Data were further analysed for species assemblages by non-metric multidimensional scaling (nMDS) in two-three dimensions with invasion status (control, invaded) as factor using PRIMER V.7 software. nMDS was used to ordinate the similarity of data between site categories (invaded, control) based on Bray-Curtis dissimilarity matrix following log-transformation of species abundance data due to zero species count in some plots. The range of clustering of sites and locations in response to invasion were assessed by analysis of similarity (ANOSIM) and similarity percentage (SIMPER). ANOSIM relates mean difference of ranks between and within groups, generating the Global statistic (R). The values of Global statistic (R)

range from -1 to +1. Values near 0 and negative values demonstrate similarity among groups. Values impending +1 indicate a strong dissimilarity among groups (Clarke and Warwick, 2001; Qureshi et al., 2019). SIMPER identified species contributed most to average dissimilarity between groups (invaded and control plots). This technique calculates average impact of each species contributing to dissimilarity between groups (Clarke and Warwick, 2001). Values of percentage similarity between groups range between 0 to 100, with 100 stating maximum similarity.

### 3. Results

To assess sampling completeness, rarefaction curves plotting cumulative number of species as a function of sampling effort were used which indicated that sampling was reasonably complete (Figure 3). A total of 66 plant species from 59 genera were documented during the study (Supplementary file). A total of 56 species were recorded in control plots compared with 37 in infested plots. Mean species diversity and richness/quadrat was higher in control plots (Figure 4).

Comparisons of ecological indices showed significant differences across sites and invasion status (Table 1). *Lantana* invasion exhibited variable impact in five sites by reducing species number per plot (S) and abundance (N) by a maximum of 46% in Chakwal. Control plots harbored on average  $13.90 \pm 3.50$  (mean  $\pm$  SD,  $n=30$ ) species. This was by  $1.734 \pm 0.14$  more than invaded plots and the difference was significant ( $t=2.27$ ,  $df=29$ ,  $p=0.00$ ). In total, 212 and 139 individuals were recorded in control and invaded plots respectively. Similarly, abundance in control and invaded plots differed by  $2.3 \pm 1.80$  (mean  $\pm$  SD,  $n=30$ ) and the difference was significant ( $t=4.08$ ,  $df=29$ ,  $p=0.00$ ). Control plots also exhibited higher values of species richness by a difference of  $0.15 \pm 0.41$ , species evenness by  $0.019 \pm 0.12$ ; Shannon index of diversity by  $0.20 \pm 0.40$  and Simpson index of dominance by  $0.22 \pm 1.27$  (Table 2).

For individual district, native flora differed significantly in species density (S), abundance per plot (N), species evenness (J') and Simpson index of dominance ( $\lambda$ ) but not in overall species richness (R) and Shannon index of diversity (H'). *Lantana* invasion had significant impacts on all ecological indices except species evenness (J') at

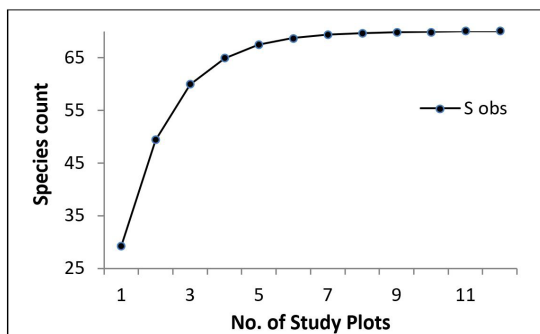


Figure 3. Rarefaction curve showing cumulative number of species recorded as a function of sampling effort.

Table 1. Summary ANOVA of invasion impacts and site on diversity indices of local plant community.

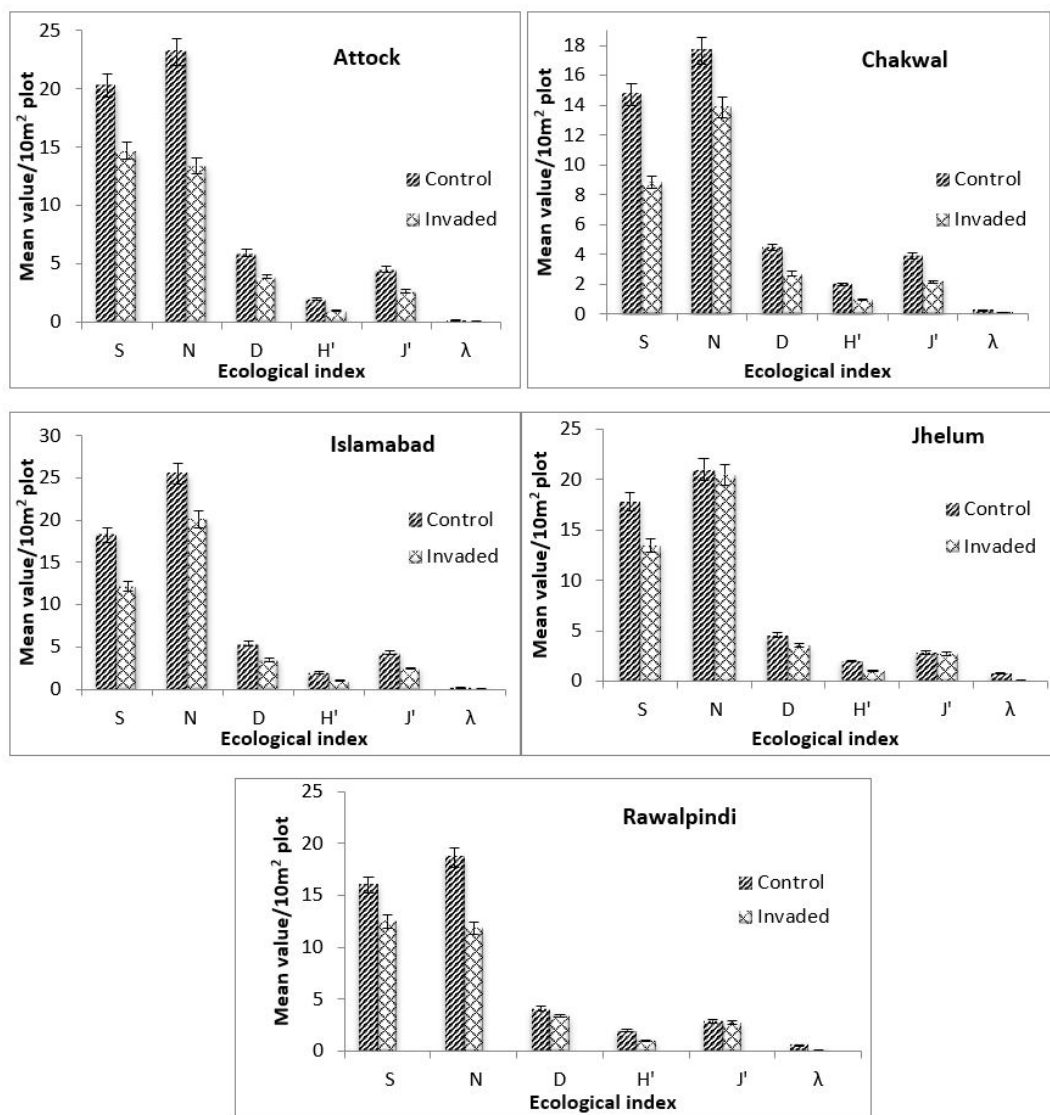
Ecological index	SUMMARY ANOVA			Mean ( $\pm$ SD)	
	Site (S)	Invasion status (IS)	S*IS Interaction	Control (30)	Invaded (30)
No. of species (S)/10m <sup>2</sup>	***	***	***	13.90 $\pm$ 3.50	12.166 $\pm$ 2.78
Abundance (N)/10m <sup>2</sup>	***	***	***	17.6667 $\pm$ 1.75	16.66 $\pm$ 2.50
Species Richness (R)	***	***	***	3.74 $\pm$ 0.72	2.7 $\pm$ 0.91
Species evenness (J')	***	***	**	0.98 $\pm$ 0.005	0.92 $\pm$ 0.25
Shannon index of diversity (H')	***	***	***	2.56 $\pm$ 0.27	1.56 $\pm$ 0.65
Simpson index of dominance ( $\lambda$ )	***	***	***	0.27 $\pm$ 0.23	0.17 $\pm$ 0.08

$\pm$ SD indicates 'standard deviation'; \*\*\*  $P \leq 0.001$ ; \*\*  $P \leq 0.02$ .



site 1 (Attock). For site 2 (Chakwal), only species richness was affected significantly. For site 3 (Islamabad) invasion impacts were not significant on native species evenness while all ecological indices were significantly affected for site 4 (Jhelum) and site 5 (Rawalpindi) (Table 2).

The ordination (nMDS) and ANOSIM showed significant magnitude of differences between invaded and control plots in all sites with global R values of 1.00 (p=0.002), 0.974 (p=0.002), 0.728 (p=0.002), 0.983 (p=0.002) and 0.930 (p=0.002) for Attock, Chakwal,

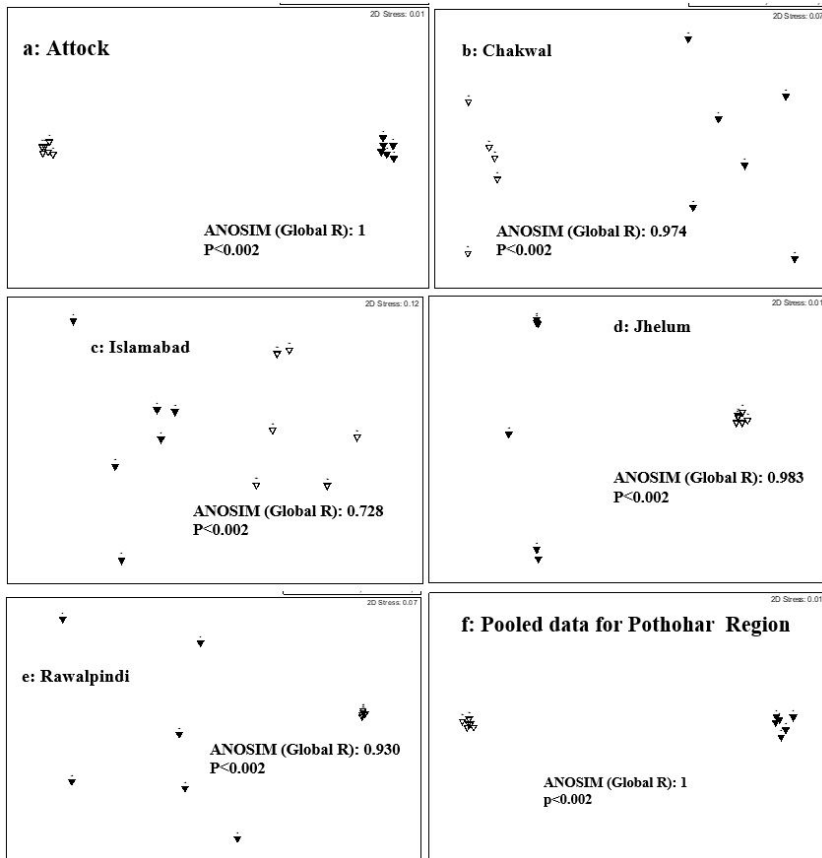


**Figure 4.** Mean values/10m<sup>2</sup> for ecological indices of invaded vs control plots in different sites. (S=Number of species; N=Abundance; D=Species richness; H'=Shannon index of diversity; J'=Species evenness; λ=Simpson index of dominance).

**Table 2.** Student's t-test for significance of differences between control and invaded plots.

Site	Number of species (S)	Abundance (N)	Margalef's index of Richness (R)	Species Evenness (J')	Shannon index of Diversity (H')	Simpson index of dominance (λ)
Attock	***	***	***	NS	**	***
Chakwal	NS	NS	**	NS	NS	NS
Islamabad	**	**	**	NS	**	**
Jhelum	***	***	***	***	***	***
Rawalpindi	***	***	***	**	***	***

\*\*\* P ≤ 0.001; \*\* P ≤ 0.02.



**Figure 5.** Multidimensional scaling (MDS) ordination and analyses of similarity (ANOSIM) results of invasion status data for Pothohar region, Pakistan; closed symbols are representative of invaded sites while open for control ones.

**Table 3.** SIPMER analysis of *Lantana* invaded and control sites in Pothohar region, Pakistan.

Average dissimilarity = 65.56%					
Species	Control	Invaded	Av. Diss.	Diss/SD	Contribution (%)
<i>Stellaria media</i> (L.) Vill.	3.04	1.71	1.38	7.99	2.10
<i>Oxalis corniculata</i> L.	2.98	0.00	1.35	9.94	2.06
<i>Cynodon dactylon</i> (L.) Pers.	2.81	1.82	1.27	9.48	1.94
<i>Digitaria ciliaris</i> (Retz.) Koeler	2.74	0.00	1.24	6.40	1.89
<i>Malva parviflora</i> L.	2.70	0.00	1.22	7.69	1.86
<i>Croton tiglium</i> L.	2.65	1.77	1.20	9.38	1.83
<i>Eclipta prostrata</i> (L.) L.	2.65	0.18	1.19	12.44	1.82
<i>Clematis grata</i> (Wall.) Kuntze	2.54	1.62	1.15	6.88	1.76
<i>Chenopodium album</i> L.	2.46	2.35	1.12	4.51	1.71
<i>Calotropis procera</i> (Aiton) W.T.Aiton	2.43	0.01	1.11	5.98	1.69
<i>Medicago sativa</i> L.	2.41	1.23	1.09	7.67	1.67
<i>Achyranthus aspra</i> L.	2.40	2.14	1.08	5.91	1.65
<i>Solanum nigrum</i> L.	2.38	2.31	1.06	9.35	1.64
<i>Datura stramonium</i> L.	2.37	2.18	1.07	9.14	1.63
<i>Sonchus asper</i> (L.) Hill.	2.25	1.49	1.02	8.80	1.55
<i>Digera muricata</i> (L.) Mart	2.15	2.21	0.98	6.44	1.49
<i>Bergenia ciliate</i> (Haw.) Sternb.	2.15	2.01	0.97	6.11	1.48
<i>Anagallis arvensis</i> L.	2.16	1.49	0.97	2.17	1.48
<i>Cannabis sativa</i> L.	2.12	2.38	0.96	6.36	1.46
<i>Portulaca oleracea</i> L.	2.11	2.41	0.96	3.12	1.46

Values are average abundance ranking (1-rare; 2-common; 3-very common; >4-dominant); Av. Diss. = Average dissimilarity; Diss/SD = Dissimilarity standard deviation.

Islamabad, Jhelum and Rawalpindi, respectively (Figure 5). The greatest dissimilarity between invaded and control plots was noticed by Attock.

Similarity percentage (SIMPER) analysis of data suggested species contributing most to average dissimilarity between control and invaded groups. This analysis also computed average contribution of species causing dissimilarity. Few top species separating invaded plots from non-invaded plots (control) for analysis are enlisted in Table 3. *Stellaria media*, *Oxalis corniculata*, *Cynodon dactylon*, *Digitaria ciliaris*, *Malva parviflora*, *Croton tiglium*, *Eclipta prostrata*, *Clematis grata*, *Chenopodium album*, *Calotropis procera*, *Medicago sativa*, *Achyranthus aspera*, *Solanum nigrum*, *Datura stramonium* and *Sonchus asper* were top contributing species causing difference between control and invaded plots.

#### 4. Discussion

*Lantana* weed is predominant in some countries in the world including Pakistan, Australia, India, and Africa (Goncalves et al., 2014). *Lantana* weed exerts impact on natural communities by displacing native species hence exert imbalance in natural ecosystems. Ecologically diversified adaptability of *L. camara* allows its rapid expansion resulting in reduction of native biodiversity and its monoculture formation. The chances of invasiveness of *Lantana* are high in future due to its rapid spread, high adaptability to different environments, tenacious resistance to cutting and burning and climate change (Taylor et al., 2012; Zhang et al., 2014). Measuring the impact of invasive species on native diversity by comparing invaded and non-invaded plots/sites collate large data sets, which give idea of variation in response of invaded community over a wider range of environments. However, this approach brings about some uncertainty over the character of invading plots prior to the invasion, i.e. to what extent are they comparable with uninvaded control plots as the plots may differ in factors other than the invasion (Hejda et al., 2009). In current study, the non-invaded plots were selected to be in close vicinity of the invaded stands with habitat conditions matching as closely as possible.

In present study, comparisons of ecological indices across invaded and control plots in Pothohar region (taken as biodiversity hotspot) of Pakistan indicated less diversity in invaded plots. These findings are in-line with other studies on alien invasive weeds, which indicated strong effects of the invader on ecosystem properties (Riaz and Javaid, 2011; Riaz and Javaid, 2010; Shabbir and Bajwa, 2007). In Pakistan, *Lantana* invasion is reported earlier from Rawalpindi and Islamabad (Malik and Husain, 2006; Fatimah and Ahmad, 2012; Khan et al. 2010). In current study, comparisons of ecological indices across invaded and control plots in Pothohar region indicated significant differences in ecological diversity indices. These findings are consistent with other studies on this invasive species, which indicated its strong effects on ecosystem properties (Lemma et al., 2015; Tadesse et al., 2017). Phenotypic

plasticity, high reproductive potential, immunization to grazing pressure, allelopathy and fire tolerance contributes to invasiveness of *Lantana* (Bhakat and Maiti, 2012).

The results demonstrate differences in vegetation composition of invaded and control plots. Analysis of variance among invaded and control plots showed significant decrease in ecological indices across site and invasion status. These findings are consistent with other studies on invasive species indicating strong negative effects of invasive species on floral diversity and ecosystem properties (Manchester and Bullock, 2000; McNeely, 2001; Grice, 2006; Borokini, 2011; Jeschke et al., 2014; Panetta and Gooden, 2017).

*Lantana* invasion exhibited variable impacts in five sites (districts) by reducing species number per plot (S), abundance (N), species richness (R), species evenness (J'), Simpson index of dominance ( $\lambda$ ) and Shannon index of diversity (H'). The trend of decrease in ecological indices in invaded plots is similar to invasion studies on *L. camara* from Australia (Duggin and Gentle, 1998), Fiji (Taylor and Kumar, 2014), Eastern Africa (Shackleton et al. 2017), South Africa (Vardien et al., 2012), China (Fan et al., 2010), Ethiopia (Chanie and Assefa, 2015) and India (Dobhal et al., 2011; Priyanka and Joshi, 2013). In current study, we noticed negative effects of *L. camara* on all of ecological indices in invaded over control plots. The highest impact is noticed in Attock district.

The ordination (nMDS) and ANOSIM showed significant magnitude of differences between diversity indices of invaded and control plots. The difference was significant for all of five study sites but greatest dissimilarity between invaded and control plots were noticed by Attock. Invasion of *L. camara* was reported earlier as top invasive species from Attock region along two other species, viz. *Prosopis juliflora* and *Xanthium strumarium* (Malik and Husain, 2006). SIMPER analysis showed 65.56% overall dissimilarity among invaded and control plots. Analysis showed herbs to be most affected by *Lantana* invasion than shrubs and trees. These were *Solanum nigrum*, *Parthenium hysterophorus*, *Ajuga bracteosa*, *Rumex dentatus*, *Typha domingensis*, *Malva parviflora*, *Tribulus terrestris* and *Oxalis corniculata*. There are related studies reporting diversity loss due to *Lantana* invasion (Sharma et al., 2009; Gooden et al., 2009; Singh et al., 2014).

#### 5. Conclusions

The increased occurrence of invasion around the world poses a major threat to native diversity. Plant invasions in novel areas deplete species diversity, alters native community composition, affect ecosystem process and thus cause huge ecological and economic imbalance. Invasive species studies in the past revealed that the effects of invasion are complex and can permanently alter the function and structure of communities, cause local annihilations and changes in ecosystem processes. Invasion by alien plant species affect the composition and dynamics of species on a wide scale and have great impact on ecosystem

functions. The decrease in ecological diversity indices in invaded over control sites in present study indicated that plant communities become less productive due to *Lantana* invasion hence a threat to plant diversity of invaded areas. There is urgent need of appropriate control measures including use of proven biological control agents for this weed in Pakistan.

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