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IMPACTS OF *XANTHIUM STRUMARIUM* L. INVASION ON VASCULAR PLANT DIVERSITY IN POTHWAR REGION (PAKISTAN)

Qureshi H.[1,2*], Anwar T.[1], Arshad M.[1], Osunkoya O.O.[3], Adkins S.W.[2]

[1]*Department of Botany, Pir Mehr Ali Shah Arid Agriculture University, Rawalpindi-46300, Pakistan*
[2]*School of Agriculture and Food Science, The University of Queensland St. Lucia-4072 Brisbane, Australia*
[3]*Invasive Plant & Animal Science, Biosecurity Unit, Queensland- Department of Agriculture & Fisheries, Brisbane-4001, Australia*
**Corresponding Author: Telephone:* +923158253252; email: humaqureshi8@gmail.com

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Abstract – Studies on the impacts of alien plants help to understand extent of biological invasion. Multiple analyses of diversity parameters at different locations allow general explanations of impact on species diversity and richness in plant communities. The current study assessed impact of *Xanthium strumarium* invasion on native plant diversity in Pothwar region of Pakistan. The approach used for study was random samplings with two categorical factors: invaded and non-invaded plots under same habitat conditions. Differences in species frequency (N), species richness (R), evenness (J'), Shannon diversity index (H') and Simpson index of dominance (λ) were compared between invaded and control plots by t-test series. Control plots harbored by average 1.3 more species/10m^2. The control category was more diverse (H'=2.00) than invaded category (H'=1.82). Non-invaded plots showed a higher floristic richness than invaded ones. At multivariate scale, ordination (nMDS) and ANOSIM showed significant magnitude of differences between invaded and control plots. The decrease in diversity indices in invaded over control sites indicated that plant communities become less biodiverse due to *Xanthium strumarium* invasion. This makes *X. strumarium* a candidate of consideration for appropriate control measures.

Keywords: Alien species, biological pollution, diversity indices, Conservation strategies, Multivariate analysis

Introduction

Species invasion is a form of biological pollution defined as 'impact of alien species on ecological services/quality' (Charles & Dukes, 2007). It includes modifications/deterioration of habitats, competition with and replacement of native species, spread of pathogens and genetic alteration within population (Alpert, 2006). Once introduced into new ecosystems, exotic taxa can interfere with natives and reduce biodiversity of natural communities (Dogra et al., 2009). Successful invasion of plants in new habitats is facilitated by their ability to colonize disturbed habitats, rapid growth and reproduction, short life cycle, production of large quantities of seeds, vegetative propagation, early flowering and seeding, different phenology from natives and pest and disease-resistance. Recently secondary metabolites are approved for their ecological significance in biological interactions (Balezentiene, 2015). Invasive plants tend to compete for space, light and nutrients more than native and therefore colonize to form monotypic stands hence wipe out native flora (Tilman, 1997). They alter ecosystem structure and function (Odat et al., 2011). Ecological assessment of invasion effects is of fundamental importance in conservation and maintaining native biodiversity.

Common Cocklebur (*Xanthium strumarium* L.) is an annual herb native to North and South America (Figure 1). It was introduced to Pakistan from Afghanistan during the Afghan war

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in early 1980s. Massive migration of Afghan nomads and their livestock resulted in small to large patches of this aggressive weed. Spiny fruits of plant clinging to wool of sheep/goats have been major force of its spread. Now it is ubiquitous weed found in orchards, agricultural and wastelands (Hashim and Marwat, 2002). Reduced biodiversity, negative effects on agricultural yield of row crops (soybean, cotton, maize and groundnut), host of crop pathogens, cattle poisoning and contamination of sheep wool by lodging of burs are adverse effects related to this weed. Facilitated dispersal of prickly burs by adhering to human clothing and animals, by water, as contaminant of wool, viability of seeds up to five years, photo-insensitivity and allelopathy are traits related to invasiveness of the weed (Hussain et al., 2013; Qureshi et al., 2014). The major allelopathic compounds found in *X. strumarium* are: xanthinin, xanthumin, xanthatin, xanthostrumarin, atractyloside, carboxyatractyloside; phytosterols, xanthanol, isoxanthanol, xanthinosin, 4-oxo-bedfordia acid, hydroquinone, xanthanolides, caffeoylquinic acids, α and γ-tocopherol, thiazinedione, deacetyl xanthumin, carboxyatractyloside, linoleic acid and several sesquiterpene lactones (Kamboj & Saluja, 2010). *X. strumarium* weed is predominant in some countries in the world including Pakistan, Australia, India, America and Turkey (Shafique et al., 2007); its presence and invasiveness in Pakistan were also reported earlier in Islamabad (Khan et al., 2010); North-west Pakistan (Marwat et al., 2010); Khyber Pakhtunkhwa (Khan et al., 2011); upper Indus plains in Punjab (Malik et al., 2012). Nowadays this species is one of the worst weeds present in Pakistan.

No previous study is reported from Pothwar region regarding *X. strumarium* diversity impacts. Current study was carried out to assess: (1) extent of invasion impact of this weed on diversity indices in different districts of Pothwar region; (2) if invasion impacts on vascular plant diversity differ between different sites (districts) in the area.

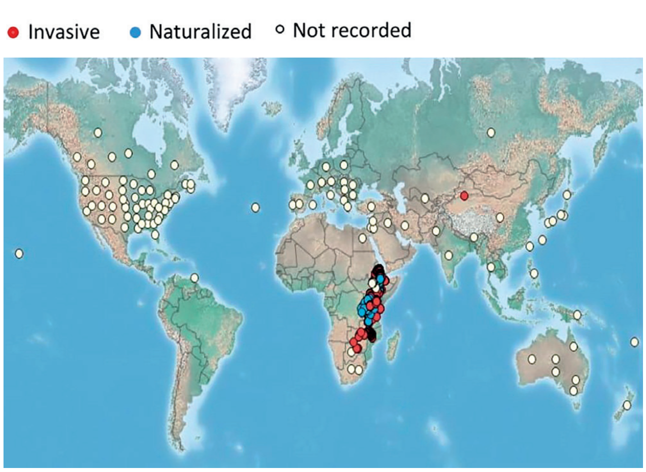


Figure 1. Distribution map and invasion status of *Xanthium strumarium* around the globe.

Materials and Methods

Study Area

The Pothwar is north-eastern plateau in Pakistan, making the northern part of Punjab. It edges Azad Kashmir (the western parts) and Khyber Pakhtunkhwa (southern parts). Pothwar Zone extends from 32.5˚N to 34.0˚N Latitude and 72˚E to 74˚E Longitude, with elevation of 350 to 575 m a.s.l. and lies between Indus and Jhelum River. The plateau expanses from salt range northward to the foothills of Himalayas. In the region four types of soil are widespread: loess, river alluvium, residual and piedmont alluvium.The Pothwar region embraces Jhelum, Islamabad, Attock, Rawalpindi, and Chakwal districts. Total area of Pothwar region is 28488.9 Km^2 (Rashid & Rasul, 2011). The climate is highly continental with hot summers and cold winters. It is divided into four seasons: Cold (December-March), Hot (April-June), Monsoon (July-September) and Post-Monsoon season (October-November). The climate data were sourced from Pakistan Meteorological Department University Road Karachi, Pakistan. The area experienced 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 advent of rains being coldest in January (14.62-18.7°C). Average temperatures range from 14°C in January to 37°C in June (Figure 2). Due to heterogeneous climate and complex geomorphology with hills and plains, Pothwar region is rich in biodiversity. *Albizia lebbeck* (L.) Benth., *Acacia modesta* Wall., *Abies pindrow* (Royle ex D. Don) Royle, *Cassia fistula* L., *Cedrela toona* Roxb. ex Rottler, *Dalbergia sissoo* Roxb., *Dodonaea viscosa* Jacq., *Ficus religiosa* L., *Ficus benghalensis* L., *Melia azedarach* L., *Olea cuspidata* Wall. Ex G. Don., *Zizyphus jujuba* Mill. and *Zizyphus nummularia* (Burm.f.) Wight & Arn. are the most common woody species in the region (Shabbir et al., 2012; Ghufran et al., 2013).

Experimental Design

Field work was carried out during July-August, 2016. The effect of *X. strumarium* invasion was studied in five districts (Attock, Chakwal, Jhelum, Islamabad & Rawalpindi) of Pothwar region. The sampling technique was random sampling. For each district, six invaded and six non-invaded paired vegetation plots (each 3.16×3.16m in size, i.e., $10m^2$ in area) were sampled. During sampling, number of individuals for each species within each plot was recorded. For grasses, each tiller was taken as separate individual. Based on visual observations, plot of invaded vegetation ('invaded plot') where the invader showed visual dominance was considered as 'treatment' and a second vegetation plot, 0.5-1 km apart

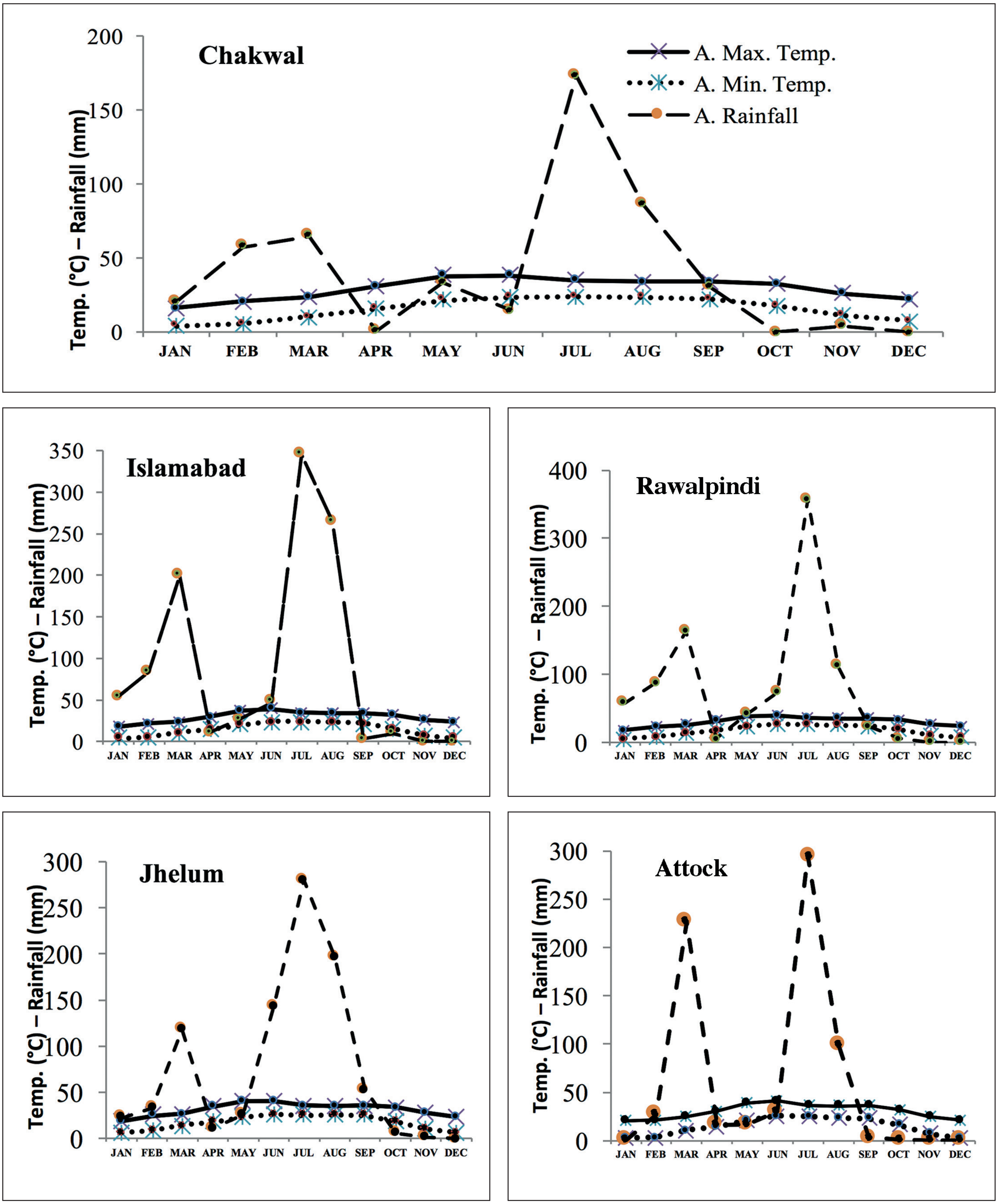


Figure 2. Mean monthly Temperature and Rainfall data for Pothwar region, Pakistan (Sourced from Pakistan Meteorological Department University Road, Karachi).

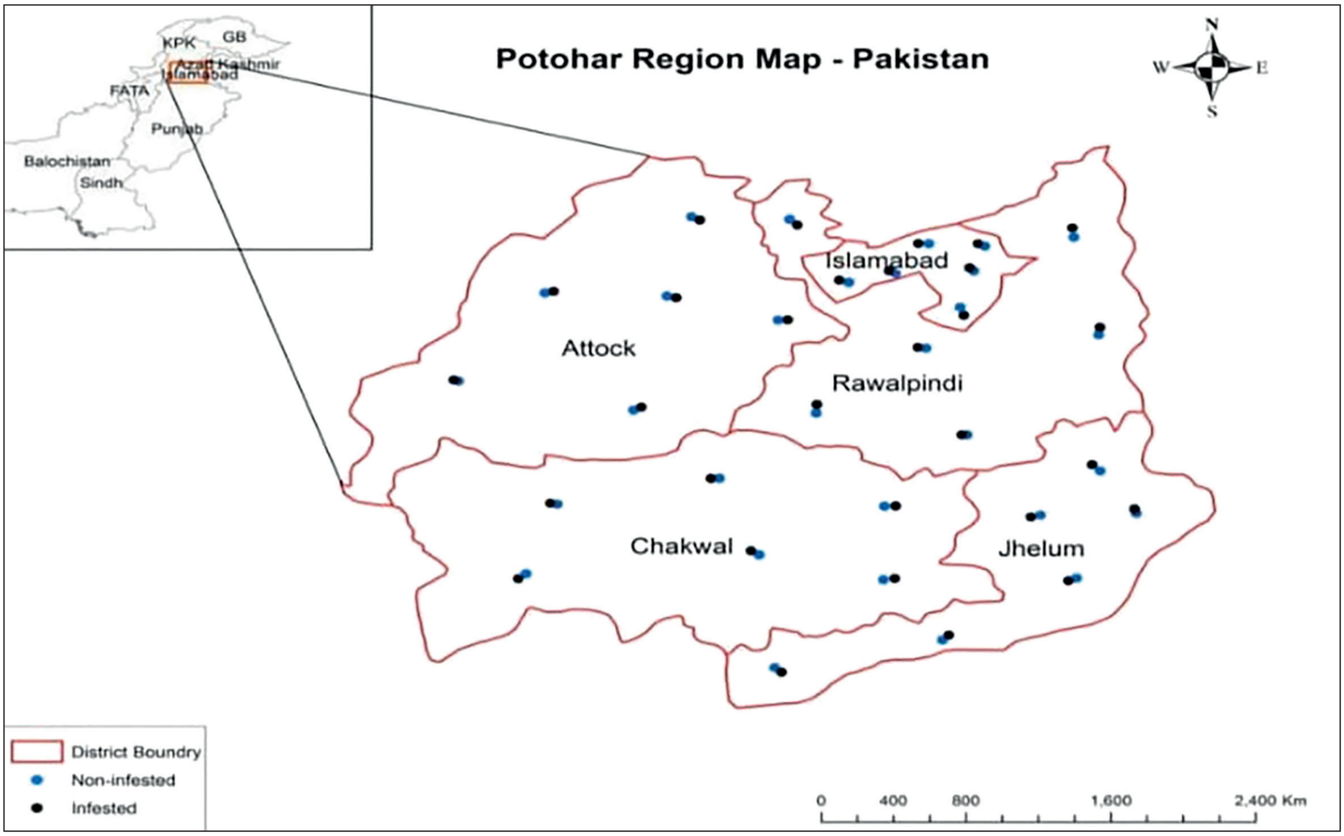


Figure 3. Distribution of sampling plots for impact analysis of *Xanthium strumarium* in Pothwar region.

from treatment, where the invader has no dominance ('non-invaded plot') was considered as the "control". In order to compare invaded plot with control one, species presence/absence data were constructed and processed in PRIMER v. 7. In all, 60 vegetation plots were sampled (consisting of six paired samples per district, and hence 30 treatments; 30 controls for the entire Pothwar region) (Figure 3). Within each randomly chosen plot, all vascular plant species in control and invaded plots were identified at species level. Diversity indices for the invaded vs. control plots were calculated and compared at various sites.

Data Analyses

Invasion impacts of *X. strumarium* on native species were assessed by calculating and comparing diversity indices including Margalef's index of richness (R), Shannon–Weaver index of diversity (H´), Simpson index of dominance (λ) and index of evenness (J') for control and invaded sites (Magurran, 1998). Rarefaction curves were plotted to determine if sampling was adequate in each district using observed, Coleman's, Jackknife, Bootstrap and Chao2 models in PRIMER v. 7 (Clarke & Warwick, 2001). The performed analysis gave comparable results; consequently

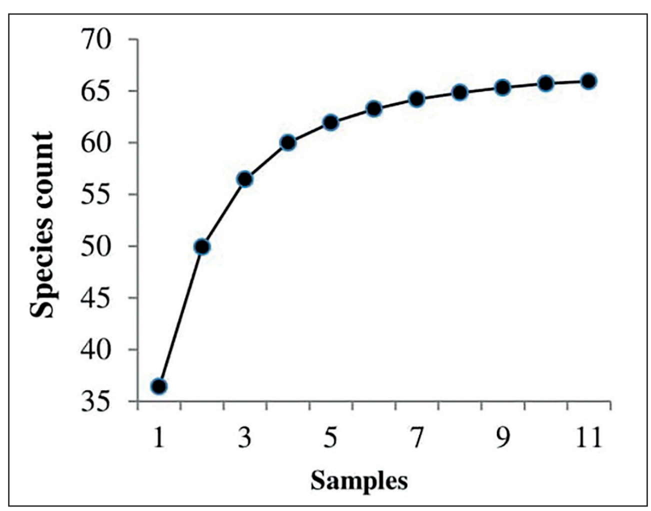


Figure 4. Rarefaction curve showing cumulative number of species recorded as a function of sampling effort.

only those of real (observed) data are presented. The presence absence data for species in each plot were subjected to univariate and multivariate analyses of non-metric multidimensional scaling procedure (Clarke & Gorley, 2015). Data were log transformed to achieve criteria of normality (evenness and

Simpson index of diversity). For invasion impact analysis, diversity indices including species frequency in terms of common or rare species, species richness (R), species evenness (J'), Shannon index of diversity (H′) and Simpson index of dominance (λ) were calculated for control as well as for invaded plots. The above diversity indices were subjected to analysis of variance (ANOVA) with invasion status and districts as factors using IBM SPSS v. 21 (Qureshi et al., 2018). Difference between the values of each index was individually tested for significance by multiple comparisons tests of t-test. Data were analyzed 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 data. 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 & Warwick, 2001; Osunkoya et al*.,* 2017). SIMPER analysis identified species contributing to average dissimilarity between groups (invaded and control plots). This technique calculates average impact of each species contributing to dissimilarity between groups (Clarke & Warwick, 2001). Higher SIMPER values indicated a higher dissimilarity.

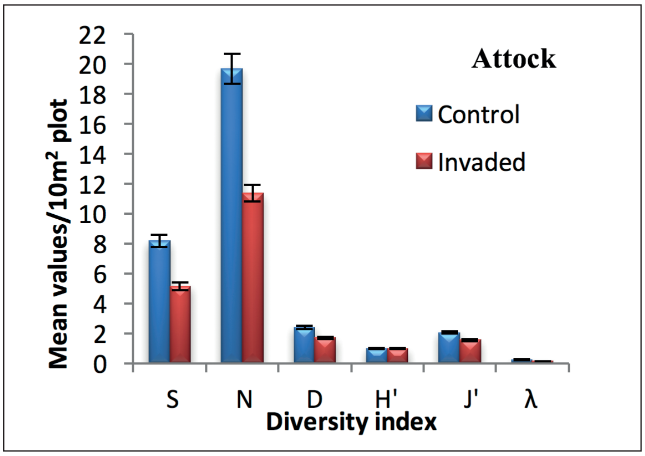
Results

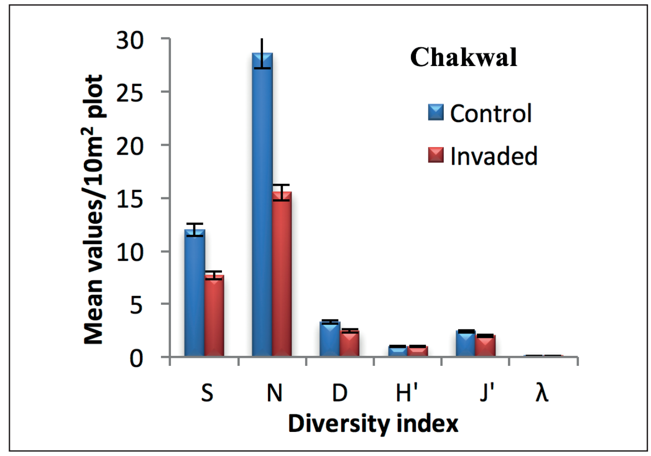
Rarefaction curves plotting cumulative number of species indicated that sampling was reasonably complete (see Figure 4). A total of 64 plant species from 59 genera were documented during the study (Table I).

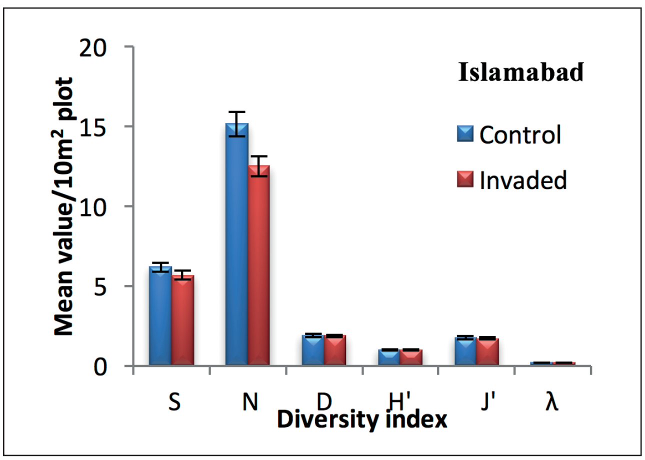
A total of 54 plant species were recorded in control plots compared with 42 in invaded plots. In total, 226 and 140 individuals of all species were recorded in control and invaded plots respectively. Mean species diversity and richness/quadrat was higher in control plots (see Figure 5). Comparisons of diversity indices showed significant difference ($p<0.05$) across sites and invasion status (Table 2). *X. strumarium* invasion exhibited variable impacts in five sites (districts) by reducing species number per plot and species frequency by a maximum of 46% in Chakwal. Control plots harbored on average 10.86±2.50 (mean±SD, n=30) species. This was by 2.86±2.07 more than invaded plots and the difference was significant ($t=-2.27$, $df=29$, $p=0.00$). Similarly,

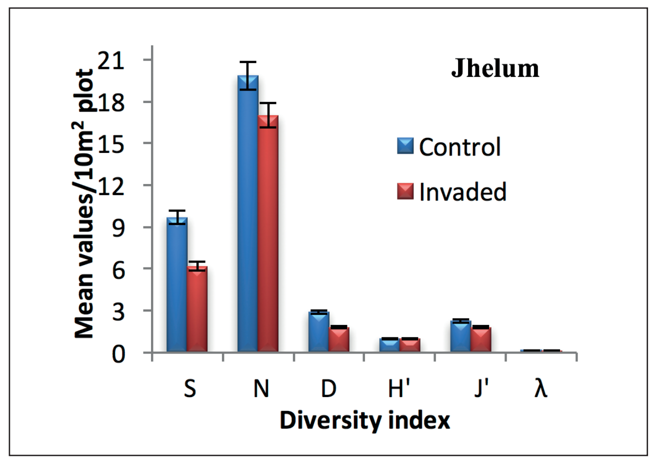
Table 1. Plant species found in the studied plots, family and growth form.

S. #	Plant Species	Family	Type
1	*Acacia nilotica* (L.) Delice	Mimosaceae	Tree
2	*Ajuga bracteosa* Wall.	Labiateae	Forb
3	*Albizia lebbeck* (L.) Benth.	Mimosaceae	Tree
4	*Amaranthus viridis* L.	Amaranthaceae	Forb
5	*Anagallis arvensis* L.	Primulaceae	Forb
6	*Artemisia scoparia* Waldst. & Kit.	Asteraceae	Forb
7	*Asparagus adscendens*	Asparagaceae	Shrub
8	*Astragalus scorplurus* Bunge.	Papillionaceae	Forb
9	*Barleria cristata* L.	Acanthaceae	Shrub
10	*Boerhavia procumbens* Banks ex Roxb.	Nyctaginaceae	Forb
11	*Calotropis procera* (Aiton) W.T. Aiton	Asclepiadaceae	Shrub
12	*Cannabis sativa* L.	Cannabaceae	Forb
13	*Capparis decidua* (Forssk.) Edgew.	Capparidaceae	Shrub
14	*Cassia fistula* L.	Caesalpiniaceae	Tree
15	*Chenopodium album* L.	Chenopodiaceae	Forb
16	*Clematis grata* Wall.	Ranunculaceae	Forb
17	*Convolvulus arvensis* L.	Convolvulaceae	Forb
18	*Cotinus coggyria* Scop.	Anacardiaceae	Shrub
19	*Cynodon dactylon* (L.) Pers.	Poaceae	Grass
20	*Dendrocalamus strictus* (Roxb.) Nees	Poaceae	Grass
21	*Dicanthium annulatum* Stapf.	Poaceae	Grass
22	*Dicanthium foveolatum* (Del.) Roberty	Poaceae	Grass
23	*Digitaria ciliaris* (Retz.) Koel	Poaceae	Grass
24	*Dodonaea viscose* (L.) Jacq.	Sapindaceae	Shrub
25	*Echinochloa crus-galli* (L.) P. Beauv.	Poaceae	Grass
26	*Eclipta alba* (L.) Hassk.	Asteraceae	Forb
27	*Eragrostis cilianensis* (All.) Vign. ex Janchen	Poaceae	Grass
28	*Euphorbia clarkeana* Hook. f.	Euphorbiceae	Forb
29	*Euphorbia helioscopia* L.	Euphorbiceae	Forb
30	*Euphorbia milii* Des Moul.	Euphorbiceae	Forb
31	*Fumaria indica* (Hausskn.) Pugsley	Fumariaceae	Forb
32	*Geranium nepalense* Sweet	Geraniaceae	Forb
33	*Heliotropium strigosum* Willd.	Boraginaceae	Forb
34	*Lactuca serriola* L.	Asteraceae	Forb
35	*Lantana camara* L.	Verbenaceae	Forb
36	*Lespedeza juncea* (Linn.f.) Pers.	Papillionaceae	Forb
37	*Malva parviflora* L.	Malvaceae	Forb
38	*Malvastrum coromandelianum* (L.) Garcke	Malvaceae	Forb
39	*Opuntia monacantha* (Willd.) Haw.	Cactaceae	Shrub
40	*Otostegia limbata* (Benth.) Boiss.	Labiateae	Shrub
41	*Oxalis corniculata* L.	Oxalidaceae	Forb
42	*Parthenium hysterophorus* L.	Asteraceae	Forb
43	*Peganum harmala* L.	Zygophyllaceae	Forb
44	*Rhamnus pentapomica* Edgew.	Rhamnaceae	Shrub
45	*Rosa damascena* Mill.	Rosaceae	Shrub
46	*Rumex dentatus* L.	Polygonaceae	Forb
47	*Saccharum spontaneum* L.	Poaceae	Grass
48	*Setaria pumila* (Poir.) Roemer & Schultes	Poaceae	Grass









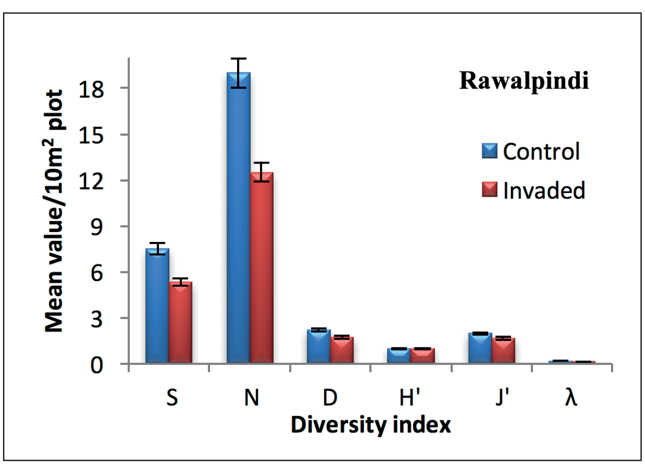


Figure 5. Mean values/10m^2 for diversity indices of invaded vs control plots in different sites (S=Number of species; N=Frequency; D=Species richness; H'=Shannon index of diversity; J'=Species evenness; λ=Simpson index of dominance).

total species frequency in control and invaded plots differed by 2.97±3.93 (mean±SD, n=30); species richness by 0.89±0.53, species evenness 0.04±0.06, Shannon index of diversity 0.90±0.29 and Simpson index of dominance by 0.18±0.09 (see Table 2). For individual sites, *X. strumarium* invasion had significant impacts on diversity indices except species richness and evenness (J') at site 2 (Chakwal). For site 3, (Islamabad) none of diversity index was affected significantly. For site 4 (Jhelum) invasion impacts on species frequency and evenness were not significant. Species evenness (J') was non-significant for site 5 (Rawalpindi) while for site 1 (Attock) all diversity indices were significantly affected by *X. strumarium* invasion (Table 3).

The ordination (nMDS) and ANOSIM showed significant magnitude of differences between diversity indices of invaded and control plots in all sites with global R values of 0.537 (p=0.002), 0.909 (p=0.002), 0.307 (p=0.0028), 0.417 (p=0.002) and 1.00 (p=0.002) for Attock, Chakwal, Islamabad, Jhelum and Rawalpindi respectively (Figure 6). The greatest dissimilarity between invaded and control plots was noticed by Rawalpindi.

SIMPER analysis showed 53.90% overall dissimilarity among invaded and control plots. Top species causing the differences between invaded and control plots are: *Solanum nigrum*, *Parthenium hysterophorus, Ajuga bracteosa, Rumex dentatus, Typha domengensis, Malva parviflora, Tribulus terrestris* and *Oxalis corniculata* (Table 4).

Table 2. Summary ANOVA of *Xanthium strumarium* invasion status in different sites of Pothwar region (Pakistan).

Ecological index	SUMMARY ANOVA			Mean (±SD)	
	Site (S)	Invasion status (IS)	S×IS Interaction	Control (30)	Invaded (30)
No. of species (S)/10m²	*	***	**	10.86±2.50	8±1.65
Frequency (N)/10m²	*	***	**	22.1±3.81	19.13±4.12
Species Richness (R)	NS	***	*	2.58±0.59	1.69±0.47
Species evenness (J')	NS	NS	NS	0.77±0.05	0.73±0.07
Shannon index of diversity (H')	*	***	**	2.15±0.27	1.25±0.32
Simpson index of dominance(λ)	*	***	**	0.203±0.075	0.022±0.033

Table 3. Student's t-test for significance of differences between control and invaded plots at different sites.

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

*** P ≤ 0.001; ** P ≤ 0.02; * P ≤ 0.05; NS (not significant) P >0.05

Table 4. SIMPER analysis of *X. strumarium* invaded and control sites in Pothwar region. Values are average frequency ranking (1-rare; 2-common; 3-very common; >4-dominant).

			Average dissimilarity = 53.90%		
	Average frequency				
Species	Control	Invaded	Av. Diss.	Diss/SD	Contribution (%)
Solanum nigrum L.	2.93	1.03	1.73	5.58	3.21
Cynodon dactylon (L.) Pers.	2.94	1.55	1.72	9.37	3.21
Parthenium hysterophorus L.	2.69	1.61	1.58	9.91	2.93
Dodonaea viscosa Jacq.	2.59	1.35	1.51	4.16	2.81
Tamarix aphylla (L.) Karst.	2.94	1.51	1.43	2.33	2.66
Ajuga bracteosa Wall.	2.41	1.34	1.42	5.80	2.63
Rumex dentatus L.	2.16	1.48	1.25	2.17	2.32
Typha domingensis Pers.	2.70	1.44	1.13	2.57	2.10
Withania somnifera (L.) Dunal	1.95	0.90	1.12	3.28	2.09
Lantana camara L.	1.89	0.52	1.11	1.95	2.06
Malva parviflora L.	2.01	0.49	1.08	2.62	2.01
Solanum surratensis Burm. F.	1.86	0.00	1.07	1.23	1.98
Cotinus coggygria Scop.	1.89	0.84	0.96	1.19	1.78
Boerhavia procumbens Banks ex Roxb.	1.61	0.00	0.95	3.03	1.77
Dichanthium foveolatum (Del.) Roberty	1.62	1.55	0.95	1.42	1.77
Malva parviflora L.	1.61	1.04	0.92	1.36	1.73
Sorghum halepense (L.) Pers.	1.48	1.06	0.91	1.35	1.69
Tribulus terrestris L.	2.10	1.44	0.90	1.75	1.68
Solanum incanum L.	1.80	1.48	0.89	1.51	1.64
Oxalis corniculata L.	1.43	0.00	0.87	0.97	1.61

Discussion

The findings of the study are consistent with other studies on the invasive species *X. strumarium*, indicating negative effects on ecosystem properties (Lemma et al., 2015; Tadesse et al., 2017). In our case, the results demonstrated differences in values of diversity indexes of invaded and control plots (H'=2.00 for invaded category, H'=1.82 for control category). Analysis of variance among invaded and control plots showed significant decrease in diversity indices values across site and invasion status. These findings are consistent with other studies on invasive species indicating strong negative effects of these species on floral diversity and ecosystem properties (Manchester & Bullock, 2000; McNeely, 2001; Grice, 2006; Borokini, 2011; Jeschke et al., 2014; Panetta & Gooden, 2017). *X. strumarium* invasion exhibited variable impacts in the studied sites by affecting values for species frequency (N),

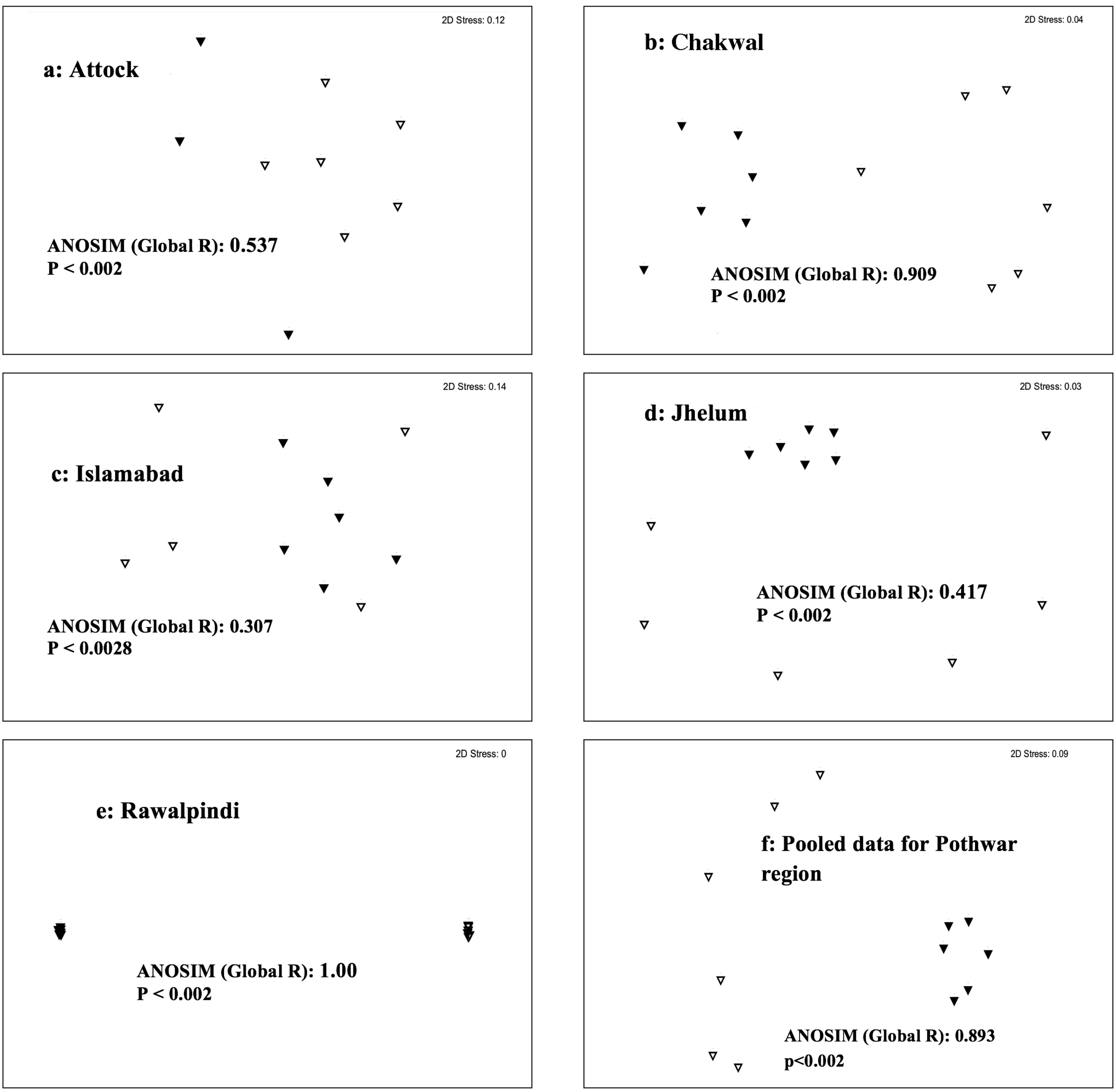


Figure 6. Multidimensional scaling (MDS) and analyses of similarity (ANOSIM) for studied plots (full symbols indicate invaded sites, empty symbols control ones).

Species richness (R), species evenness (J'), Simpson index of dominance (λ) and Shannon index of diversity (H'). The trend of decrease in values of diversity indices in invaded plots is similar to invasion studies on *X. strumarium* from Ethiopia, Zimbabwe, Pakistan, Nigeria, Tanzania and India (Lemma et al., 2015; Tadesse et al., 2017; Seifu et al., 2017; Chikuruwo et al., 2017, Hussain et al., 2014). The ordination (nMDS), ANOSIM and SIMPER analysis showed significant magnitude of differences between values of diversity indices in invaded and control plots. Invasion of *X. strumarium* was reported earlier as a top invasive species from Rawalpindi region in Pakistan along two other species, viz. *Prosopis juliflora* and *Lantana camara* (Malik & Husain 2006).

The observed negative effects of *X. strumarium* on native vegetation could be explained by its wide ecological amplitude that allows its rapid expansion resulting in the formation of monospecific stands and native biodiversity reduction. The invasiveness of *X. strumarium* will be very high in future as it is highly adaptable to different environments, highly mobile and tolerant to a variety of soil conditions (Tadesse et al., 2017). Facilitated dispersal of prickly burs by adhering to human clothing and animals, by water, as contaminant of wool, viability of seeds up to five years, photo-insensitivity and allelopathy are traits related to invasiveness of the weed (Hussain et al., 2013; Qureshi et al., 2014).

Conclusion

The increased occurrence of biological invasion around the World poses a major threat to native biodiversity. Plant invasions negatively affect species diversity, alter indigenous community composition, affect ecosystem processes and thus cause huge ecological and economic imbalance. Past studies on invasive species 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 diversity indices in invaded over control sites, resulted in the present study, indicated that plant communities become less diverse due to *X. strumarium* invasion, which is proved as a threat to plant diversity of invaded areas. There is urgent need of appropriate control measures.

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