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Seed and cone biometry of *Juniperus excelsa* from three Provenances in Balochistan

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Abstract

In present study cone and seed traits of the *J. excelsa* in three distinct regions namely Ziarat, Zarghoon Ghar and Harboi districts of Balochistan were analyzed. The aim of this study was to compare the biometrical characters of cone and seed of *J. excelsa* growing in three provenances in Balochistan with the intention to verify whether the spatial segregation had brought any variation in the provenances with respect to biometric characteristics. Inter-population differences were greater than intra-population differences with regard to cone size and number of seeds per cone. Maximum number of seeds per cone was found to be 8 and seed size was reduced with the increase in number of seeds in a cone. The percentage of the maximum number of filled seeds was higher in Ziarat. The mean values of cone diameter, cone mass and seed mass were also comparatively higher in Ziarat; while the average values of empty and dead seeds per cone were higher in Zarghoon Ghar. Elevation was found to effect on the number of filled seeds. At least 75 percent seeds were found to be empty and dead filled shrunken seeds. Infested and bored seed coat seeds were also found. The populations of Ziarat and Zarghoon Ghar varied with respect to cone and seed morphological characteristics, though they were located adjacent to each other. However, the spatially segregated populations of Ziarat and Harboi were closely related with respect to cone and seed morphological characteristics.

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Introduction

Based on the morphology *Juniperus excelsa* is divided into two subspecies namely *J. excelsa* subsp. *excelsa*, which covers mountainous and sub-mountainous areas of the Balkan Peninsula in the west, through Anatolia, Syria and Lebanon to Crimea in the north and Iran in the east; while the second subspecies *J. excelsa* sub sp. *polycarpos* (K. Koch) Takht. Is found further to Trans-Caucasia and Central-Asia.

The present research work concerns with *Juniperus excelsa* subsp. *polycarpos* which is distributed in Pakistan in some of the inner valleys of the Himalaya and Balochistan at the elevation of 1200-3000 m. Its natural stands in Balochistan, Pakistan are distributed between 20°9'N and 30°37'N and between 67°1'E, as well as in some isolated dry valleys at an elevation of 1200 m to 3000m (Rafi,1965; Nasir *et al.*, 1960).

The province of Balochistan in Pakistan encompasses approximately 141,000 hectares of *J. excelsa* forests. *J. excelsa* trees typically grow as pure stands; its forests are characteristically open and multistoried (Sheikh, 1985). *J. excelsa* is a slow-growing, monoecious or dioecious and wind-pollinated tree that can reach 20-25 m in height (Takhtajan, 1986; Farjon, 2005; Adams, 2008; Farjon, 2010).

The field reports by the foresters suggest that cones of *J. excelsa* appear in spring and ripen in September to October in second year; the cones are bluish black when ripe. Good seed-years occur at less frequent intervals.

Seedlings appear naturally to a varying extent, but the great majority perishes probably from drought. A heavy snow fall has been found to assist reproduction by increasing the moisture in the soil.

The seed and cone traits of *Juniperusexcelsa* trees growing in three separate provenances in Balochistan have been analyzed. The provenances are namely Ziarat (district Ziarat), Zarghoon (district Quetta) and Harboi (district Kalat).

The regions Ziarat and Zarghoon Ghar are located adjacent to each other although they are detached from each other, while Ziarat and Harboi are about 210km apart. The approximate location of *J. excelsa* forests in Balochistan is shown in (Fig. 1).

The three populations examined in this study are spatially disconnected; therefore the variability or similarity among the populations with regard to cone and seed biometry has been investigated. Such studies have been carried out to correlate geographical distances and morphological variability (Mazur *et al.*, 2004; Farjon, 2005).

The cone morphology and genetic diversity of *J. excelsa* has been fairly studied elsewhere in the world (Farjon, 1992; Schulz *et al.*, 2003; Douaihy *et al.*, 2011) while the comprehensive work related to the species native to Balochistan, Pakistan is rare.

Mostly the published work carried out on *J. excelsa* of Balochistan is related to the Ziarat tract of Juniper (Sarangzai *et al.*, 2012; Ahmed *et al.* 1990) and so far very limited research work has been performed to investigate the comparative morphological characteristics of cone and seed morphological variation among the three distinct provenances of Juniper in Balochistan.

Morphological data are important in the understanding of life cycles, geographic and ecologic distribution, evolution, conservation status, as well as species delimitation (Kaplan, 2001). The role of seed and cone morph metric characteristics is very significant in the reproductive biology of a species. The reproductive structures assist a species in adaptability against different hazards faced in the nature, which include granivory and damage caused by the forest fires and many others. The aim of this study is to compare the biometrical characters of seed and cone of *J. excelsa* growing in three provenances in Balochistan. The study is carried out to demonstrate whether the spatial segregation have brought any variation in the provenances with respect to biometric characteristics.

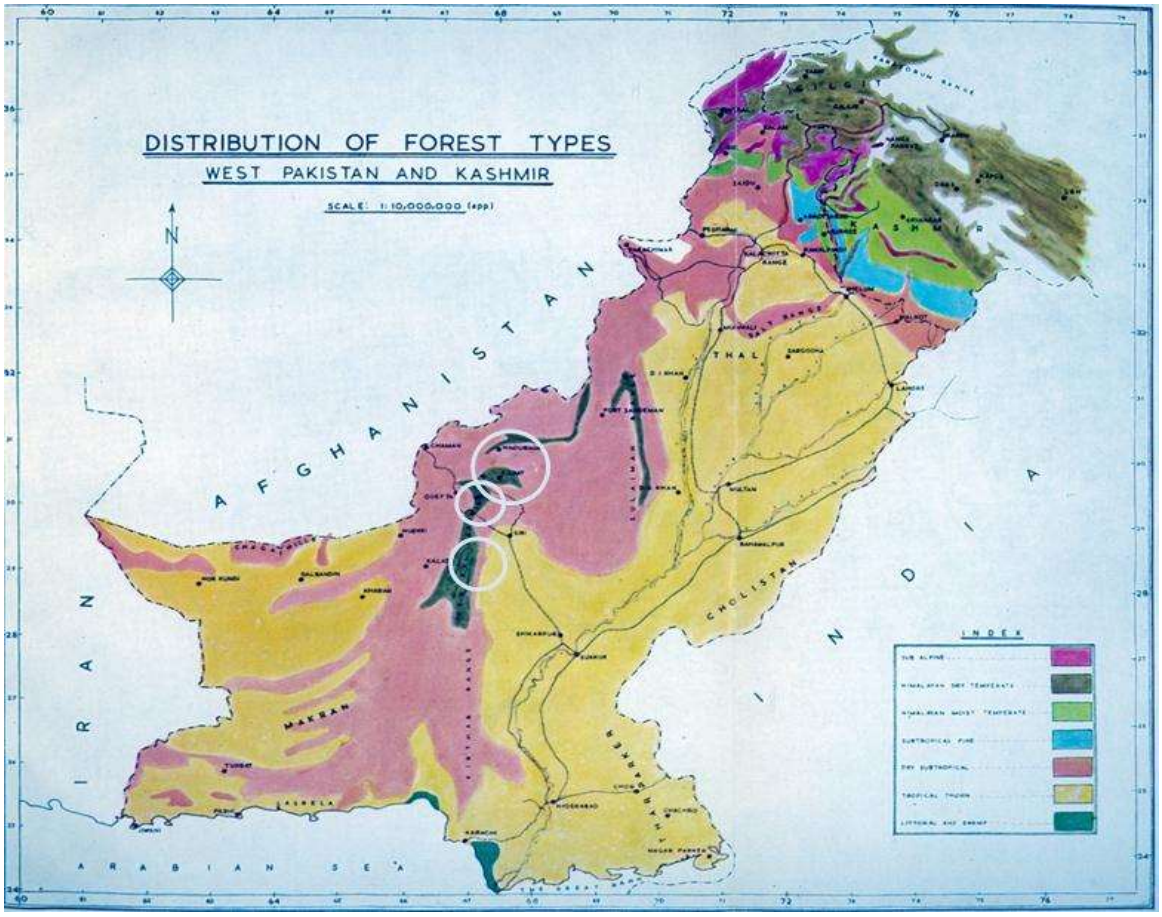

Fig. 1. The map showing the approximate location of *Juniperus excelsa* forest in Balochistan. (Champion and Khatak, 1965).

Materials and methods

Study area

The study areas Ziarat, Zarghoon and Harboi are located at (30°22′56" N 67° 43′27 E"), (30°15′54" N 67°18′55 E") and (29°02′55" N 66°43′55 E") respectively. The study area lies at elevation ranging from 2000 to 2700 m.

Plant material and characters studied

Ten populations of *J. excelsa* were sampled in the month of October from the three separate tracts of Juniper in Balochistan, Pakistan. Samples of female cones were collected at a height of about 1.0-5.0 m above ground level in each population, as described by (Mazur *et al*., 2010).

The samples were collected from southern, south-western and south-eastern expositions (Klimko *et al*., *2007*). For each sampled tree, 15-20 ripe seed cones were collected from the same part of the crown. The measurement was performed on dry material.

The provenance, population acronyms, elevation, aspect, and geographic coordinates of the populations studied are given in Table 1. Various characters studied are given in Table 2. Cone dimensions measurements were carried out with a numerical caliper (0.01mm) and the cone and seed mass was weighed to the accuracy of (±0.01g). Seed cutting tests were performed as per ISTA rules (1996).

Statistical analysis

The data obtained was statistically analyzed with IBM SPSS Statistics 20. The statistics such as arithmetic means, standard deviations and variation coefficient were calculated for the particular characters for individuals and populations. The interactions between characters were checked with Pearson's correlation coefficient. While comparison of means was done by ANOVA, pair wise multiple comparison procedures were performed with the Tukey's test.

Table 1. Provenance, population acronyms, elevation, aspect, and geographic coordinates of the *Juniperus excelsa* populations.

Provenance	Population	Elevation (m)	Aspect	Coordinates
Ziarat	ZRT 1	2700	NE	30°25´N 67°30´E,
	ZRT 2	2600	NE	30°24´N 67°30´E
	ZRT 3	2500	SE	30°23´N 67°31´E
	ZRT 4	2400	SW	30°22´N 67°31´E
Zarghoon Ghar	ZRG 1	2600	NE	30°16´N 67°19´E
	ZRG 2	2500	SE	30°15´N 67°19´E
	ZRG 3	2400	SW	30°15´N 67°19´E
Harboi	HRI 1	2200	NE	29°12´N 66°50´E
	HRI 2	2100	NE	29°12´N 66°51´E
	HRI 3	2000	SW	29°12´N 66°53´E

Results

Ten parameters of cone and seed biometric characteristics with descriptive statistics of *Juniperus excelsa* from 10 populations of three different provenances in Balochistan were recorded.

The codes of characters studied, average values with standard deviation (SD), minimum and maximum values of studied characters, confidence and variation coefficients (CV) of analyzed characters of cones and seeds of *J. excelsa* are presented in (Table 2).

Table 2. The codes of characters studied, average values with standard deviation (SD), minimum and maximum values of studied characters, confidence and variation coefficients (CV) of analyzed characters of cones and seeds of *Juniperus excelsa*.

Code	Character	Accuracy and measure	Mean± SD	Minimum	Maximum	Confidence	Variation Coefficient (%)
CD	Diameter of Cone	0.01 mm	9.21±1.19	5.50	11.00	0.01	12.92
CM	Cone mass	0.01 g	0.46±0.17	0.15	1.00	0.01	36.95
SN	Number of seeds/cone	specimen	4.05±1.14	01	08	0.04	28.14
SM	Seed mass	0.01 g	0.17±0.09	0.05	0.35	0.02	52.94
PM	Pulp mass (g)	0.01 g	0.30±0.11	0.10	0.65	0.04	36.66
SL	Length of Seed (mm)	0.01 mm	4.70±0.36	2.50	5.30	0.01	7.65
SW	Width of seed (mm)	0.01 mm	2.75±0.26	2.00	3.50	0.01	9.45
FS	Filled seeds	Specimen	0.84±0.59	0.50	0.94	0.01	70.23
ES	Empty seeds	Specimen	1.08±0.71	0.65	1.84	0.01	65.74
DS	Dead seeds	Specimen	2.12±0.93	1.74	2.54	0.02	43.86

The average values of cone diameter (CD) was found 9.21±1.19mm and variation coefficient for (CD) was calculated as (CV=12.92%) while minimum values of (CD) were measured 5.5 mm and the maximum values recorded for the (CD) were 11.00 mm among all the studied populations (Table 2). The cone diameter was found to increase with increase in number of seeds/cone as the mean largest cone

diameter was found (9.71±0.74mm) in ZRG 3 population and the highest average number of seeds (4.86±1.20) per cone were also found in the same population whereas cone mass partially depended on the percentage of filled seeds since moisture content also had bearing on cone mass.

The mean (CD) at population level ranged between 8.40-9.71 mm among different populations. Coefficients of variation for (CD) varied between 9.70 to 17.57%, 7.62 to 10.05% and 13.08 to 16.54% for populations from Ziarat, Zarghoon Ghar and Harboi provenances respectively. The average cone mass was found 0.46±0.17g (CV=36.95%) and ranged between 0.38 to 0.58g among the populations. The heaviest cone was found in Ziarat (ZRT2) population weighing 1.00g. Cones and seeds of *J. excelsa* are depicted in (Fig. 2).

Fig. 2. Cones and seeds of *Juniperus excelsa*.

The number of seeds per cone (SN) ranged between 1-7, 2-8, and 1-7 for Ziarat, Zarghoon Ghar and Harboi provenances respectively. The mean number of seeds per cone was found 4.05±1.14 (CV=28.14%) (Table 2). Mean maximum number of seeds per cone was 3.81 for ZRT2, 4.86 for ZRG3, and 4.07 for HBI1 population. Highest seed mass and pulp mass was found in ZRG 3 population. Cones from ZRG 3 population allocated 0.22 g to seed mass and 0.36 g to pulp mass which is 38% and 62% of biomass of cones respectively. However, pulp mass was found to reduce with increase in number and size of seeds in a cone.

The average seed length (SL) was found 4.70±0.36 mm with (CV=7.65%) and ranged between 2.50-5.30 mm among different populations and the seed width (SW) averaged 2.75±0.26 mm having variation coefficient (CV=9.45%) whereas the minimum and maximum values for seed width were recorded 2.00mm and 3.5 mm respectively. Seeds containing full grown embryos were relatively large in size.

Maximum number of filled seeds percentage was 25%, 19% and 21% for Ziarat, Zarghoon Ghar and Harboi populations respectively while the average number of filled seeds per cone (FS) was recorded 0.84±0.59 having a variation coefficient (CV=70.23%) which is the maximum variation coefficient among all the studied characters of cone and seed morphology of *J. excelsa*. The average minimum number of filled seeds per cone was 0.50 whereas the maximum number of filled seeds per cone was recorded 0.94 seeds per cone. Empty seeds percentage varied between 16 to 38% while dead and aborted seeds percentage was found to vary between 43 to 66% among the populations. Infested and bored seed-coat seeds were also included in empty seeds category.

The mean number of empty seeds/cone (ES) was found 1.08±0.71 with (CV=65.74%) and the minimum and maximum values for (ES) were recorded 0.65 and 1.84 seeds per cone respectively. The mean number of dead seeds per cone (DS) averaged 2.12±0.93 with a variation coefficient (CV=43.86%) and the minimum and maximum number of dead seeds/cone were recorded 1.74 and 2.54 respectively (Table 3).

Table 3. Descriptive statistics and variation coefficients (CV) of the measured morphological traits of *Juniperus excelsa* at the population level.

Population	Measured Trait	CD (mm)	CM (g)	CSN	SN	SM (g)	PM (g)	SL (mm)	SW (mm)	FS %	ES %	DS %
ZRT 1	Average	9.00	0.40	5.90	3.70	0.14	0.26	4.59	2.72	0.79	1.17	1.74
	SD	1.18	0.15	0.23	1.11	0.02	0.10	0.46	0.31	0.23	0.34	0.53
	CV (%)	13.11	37.50	3.89	30.00	14.28	38.46	10.02	11.39	29.11	29.05	30.45
ZRT 2	Average	9.48	0.52	5.90	3.81	0.19	0.33	4.80	2.80	0.94	1.04	1.83
	SD	0.92	0.18	0.23	0.98	0.06	0.11	0.35	0.28	0.24	0.26	0.47
	CV (%)	9.70	34.61	3.89	25.72	31.57	33.33	7.29	10.00	25.53	25.00	25.68
ZRT 3	Average	9.25	0.40	5.90	3.78	0.15	0.25	4.75	2.78	0.94	1.02	1.82
	SD	1.15	0.10	0.23	0.98	0.05	0.09	0.32	0.26	0.24	0.26	0.47
	CV (%)	12.43	25.00	3.89	25.92	33.33	36.00	6.73	9.35	25.53	25.49	25.82
ZRT 4	Average	8.42	0.38	5.90	3.71	0.13	0.25	4.63	2.70	0.65	1.29	1.77
	SD	1.48	0.13	0.23	1.07	0.04	0.09	0.35	0.31	0.18	0.37	0.51
	CV (%)	17.57	34.21	3.89	28.84	30.76	36.00	7.55	11.48	27.69	28.68	28.81
ZRG 1	Average	9.55	0.53	5.90	4.26	0.19	0.34	4.73	2.75	0.78	1.49	1.99
	SD	0.96	0.14	0.23	1.02	0.05	0.09	0.32	0.25	0.21	0.33	0.46
	CV (%)	10.05	26.41	3.89	23.94	26.31	26.47	6.76	9.09	26.92	22.14	23.11
ZRG 2	Average	9.70	0.52	5.90	4.41	0.19	0.33	4.78	2.71	0.86	1.58	1.97
	SD	0.93	0.17	0.23	1.13	0.06	0.11	0.33	0.24	0.22	0.40	0.52
	CV (%)	9.58	32.69	3.89	25.62	31.57	33.33	6.90	8.85	25.58	25.31	26.39
ZRG 3	Average	9.71	0.58	5.90	4.86	0.22	0.36	4.70	2.75	0.91	1.84	2.11
	SD	0.74	0.17	0.23	1.20	0.06	0.11	0.30	0.25	0.22	0.45	0.51
	CV (%)	7.62	29.31	3.89	24.69	27.27	30.55	6.38	9.09	24.17	24.45	24.17
HBI 1	Average	9.25	0.48	5.90	4.07	0.17	0.31	4.70	2.78	0.86	0.67	2.54
	SD	1.21	0.18	0.23	1.18	0.05	0.11	0.30	0.24	0.24	0.19	0.74
	CV (%)	13.08	37.50	3.89	28.99	29.41	35.48	6.38	8.63	27.90	28.35	29.13
HBI 2	Average	8.60	0.39	5.90	3.74	0.14	0.25	4.68	2.75	0.58	0.69	2.47
	SD	1.33	0.13	0.23	0.97	0.04	0.08	0.32	0.23	0.14	0.18	0.64
	CV (%)	15.46	33.33	3.89	27.95	28.57	32.00	6.83	8.36	24.13	26.08	25.91
HBI 3	Average	8.40	0.38	5.90	3.85	0.14	0.24	4.59	2.76	0.50	0.81	2.54
	SD	1.39	0.12	0.23	1.10	0.03	0.07	0.46	0.23	0.14	0.23	0.72
	CV (%)	16.54	31.57	3.89	28.57	21.42	29.16	10.02	8.33	28.31	28.39	28.34

Pearson correlation was computed to find out the relationship between various cone and seed studied parameters. Cone diameter and cone mass were positively correlated (R^2 = 0.85, $P<0.05$) while seed mass and pulp mass were significantly inversely correlated (R^2= -0.15, $P<0.02$). Seed length and seed width were also positively correlation with number of seeds per cone (R^2= 0.55, $P<0.03$) and (R^2=0.62, $P<0.04$) respectively. Pulp mass was also significantly correlated with seed mass (R^2 = -0.15, $P<0.02$). Empty seeds were found significantly correlated with seed length (R^2 =0.18, $P<0.01$).

A relative reduction in cone diameter and cone mass was found at highest altitude in ZRT 1 population as compared to ZRT2 population. ZRT3 population was closer to ZRT2 with respect to cone and seed biometric characters while at lowest altitude further decline in biometric characters was observed in ZRT4 population. The highest average cone diameter recorded in ZRG3 was (9.71mm) and cone mass was (0.58g).

Despite distantly located HBI1 was found closely related to ZRT3 population with respect to cone diameter and number of seeds/cone; however, percentage of dead-filled seeds was highest in HBI1 population. A further character similarity was recorded in the populations from Harboi (HBI2 and HBI3) with a population from Ziarat (ZRT4) (Table 3). Ziarat and Harboi provenances were also found closer to each other with respect to number of seeds/cone and percentage of filled seeds.

A one way analysis of variance (ANOVA) of the studied characters of cone and seeds of *J. excelsa* at population level from the populations of Ziarat, Zarghoon Ghar and Harboi provenances showed that all the studied characters of cone and seed within groups and between groups were statistically significantly different at ($P<0.05$). The lowest *F statistic* (2.07) was found for seed width while the highest *F statistic* (58.72) was found for empty seeds.

All the three provenances Ziarat, Zarghoon Ghar and Harboi when compared with each other with respect to cone diameter, cone mass and pulp mass were found significantly different from each other at (P<0.05). The number of seeds/cone from Ziarat, and Zarghoon Ghar were found significantly different whereas Ziarat and Harboi were not significantly different at (P<0.05). The seed length and seed width from Ziarat and Zarghoon Ghar were not significantly different from each other while filled, empty and dead seeds from each provenance when compared with each other were found significantly different at (P<0.05). Multiple comparisons of means of cone and seed characteristics of the three provenances of *J. excelsa* showed that the means of all characteristics studied were significantly different among all three provenances except seed per cone, seed length and seed width (Table 4 and 5).

Table 3. Multiple comparisons of means of cone characteristics of three provenances of *Juniperus excelsa*.

Dependent Variable	(I) Provenance	(J) Provenance	Mean Difference (I-J)
Cone Diameter (mm)	Ziarat	Zarghoon	-.4100*
		Harboi	.4958*
	Zarghoon	Ziarat	.4100*
		Harboi	.9058*
	Harboi	Ziarat	-.4958*
		Zarghoon	-.9058*
Cone mass, g	Ziarat	Zarghoon	-.1003500*
		Harboi	.0259467*
	Zarghoon	Ziarat	.1003500*
		Harboi	.1262967*
	Harboi	Ziarat	-.0259467*
		Zarghoon	-.1262967*
Seed per cone	Ziarat	Zarghoon	-.748*
		Harboi	-.122
	Zarghoon	Ziarat	.748*
		Harboi	.627*
	Harboi	Ziarat	.122
		Zarghoon	-.627*
Pulp mass	Ziarat	Zarghoon	-.0626667*
		Harboi	.0163167*
	Zarghoon	Ziarat	.0626667*
		Harboi	.0789833*
	Harboi	Ziarat	-.0163167*
		Zarghoon	-.0789833*

*. The mean difference is significant at the 0.05 level.

Table 4. Multiple comparisons of means of seed characteristics of three provenances of *Juniperus excelsa*.

Dependent Variable	(I) Provenance	(J) Provenance	Mean Difference (I-J)
Seed mass	Ziarat	Zarghoon	-.0140667*
		Harboi	.0344333*
	Zarghoon	Ziarat	.0140667*
		Harboi	.0485000*
	Harboi	Ziarat	-.0344333*
		Zarghoon	-.0485000*
Seed length	Ziarat	Zarghoon	-.0229500
		Harboi	.0574667*
	Zarghoon	Ziarat	.0229500
		Harboi	.0804167*
	Harboi	Ziarat	-.0574667*
		Zarghoon	-.0804167*
Seed Width	Ziarat	Zarghoon	.0300000
		Harboi	.0061667
	Zarghoon	Ziarat	-.0300000

Dependent Variable	(I) Provenance	(J) Provenance	Mean Difference (I-J)
Filled seed	Harboi	Harboi	-.0238333
		Ziarat	-.0061667
		Zarghoon	.0238333
	Ziarat	Zarghoon	-.123*
		Harboi	.277*
	Zarghoon	Ziarat	.123*
		Harboi	.400*
	Harboi	Ziarat	-.277*
		Zarghoon	-.400*
EpmtySeeds	Ziarat	Zarghoon	-.408*
		Harboi	.348*
	Zarghoon	Ziarat	.408*
		Harboi	.757*
	Harboi	Ziarat	-.348*
		Zarghoon	-.757*
Dead Seeds	Ziarat	Zarghoon	-.228*
		Harboi	-.738*
	Zarghoon	Ziarat	.228*
		Harboi	-.510*
	Harboi	Ziarat	.738*
		Zarghoon	.510*

*. The mean difference is significant at the 0.05 level. *n=1800.*

Discussion

In present study the data concerning the number of seeds per cone revealed that inter population differences were greater than intrapopulation difference. Ziarat and Zarghoon Ghar Juniper forests are located adjacent to each other; however, there is more character similarity like number of seeds per cone between Ziarat and Harboi provenances which are almost 210 km apart from each other. These findings are in agreement with the findings on comparative analysis of cone essential oil contents of *Juniperus excelsa* of same populations (Khajjak *et al*., 2012). The mean number of seeds per female cone found in our study was 4.02 coincidently; this number is similar to *J. thurifera*. This may be attributed to the reason that populations of *J. excelsa* are botanically and ecologically close to *Juniperus* and considered to have derived from a hypothetical common ancestor (Barbero *et al*., 1994; Jimenez *et al*., 2003). Nevertheless, these findingsdiffer from the studies on *J. excelsa*subsp. *excelsa* (Adam *et al*., 1993; Marcysiak *et al*., 2007; Douaihy *et al*., 2012). Previous studies have reported high phenotypic variability among populations of different *Juniperus* species and other out crossed, wind pollinated species with large geographical ranges (Mazur *et al*., 2004; Ward, 2007; Brus *et al*., 2010; Sultangaziev *et al*., 2010; Douaihy *et al*., 2011). Ahmed *et al*., (1990) carried out a study of *J. excelsa* in Ziarat Balochistan with regard to population dynamics of the species and reported that because of infestation a high proportion of seeds and cones were found hollow.

I this study the marginal populations being located at highest and lowest altitude exhibited a relative reduction in almost all the biometric characters studied. This may have been caused due to the cold temperatures and climate change while at low altitude degradation of forests due to human activities may possibly have been the reason. Different authors have reported similar findings like decrease in seed mass at high altitude (Cruden, 1972; Shaheen *et al*., 2011; Sarangzai *et al*., 2012). Cone diameter and number of filled seeds per cone were found positively correlated. Sometime empty seeds possess exceptionally large seed coat which has bearing on cone diameter. Producing large quantity of empty seeds has been considered an adaptation of species to deceive predators (Fuentes *et al*., 1998). Production of a large number of empty seeds is also considered due to 'delayed fertilization' of many Gymnosperms which takes place long after pollination and the onset of fruit development (Willson and Burley, 1983), poor dispersal of pollen (Huntley and Birks, 1983; Moore *et al*., 1991), resource restraint (Mohan *et al*., 1996).

Dead aborted and shrunken seeds percentage was rather greater than empty seeds in all the studied populations of *J. excelsa*. This may be because of self-pollination of ovules, aborted female gametophytes and disintegration of male gametophytes which may lead to the loss of embryos at one of their embryogenesis stages (Ahani *et al*., 2013). Non-specificity of pollination drop may be another cause of production of empty seeds in Junipers. Mugnaini *et al*., (2007) have reported that there are some factors which may lead to induce partial withdrawal of the pollination drop, which may include non-viable conspecific pollen, heterospecific pollen as well as abiotic particles of an appropriate size. This leads to a decreased surface exposed for pollen capture and results in reducing the likelihood of effective pollination. Therefore, they suggest that deposition of non-viable pollen or inorganic particles may reduce pollination efficiency in *Juniperus* by preventing viable pollen from reaching the surface of the nucellus and may have major ecological implications. Under unfavorable environmental conditions, the reproductive effort of the females could be lessened by producing low quality cones as well (Houle and Babeux, 1994). Pattern of filled seeds percentage was found to be affected with increasing altitude as Ziarat (ZRT2) having the maximum number of filled seeds and Harboi (HBI3) minimum number of filled seeds. Ortiz *et al*., (2002) reported similar findings along an altitudinal gradient in *Juniperus communis*. *Juniperus excelsa* is usually a dioecious taxon; however, monoecious trees were also found rarely. Being dioecious species the count of male and female trees and pollen ovule ratio could affect process of fertilization which results in reduced number of viable seeds (Arista and Talavera, 1996; Ortiz *et al*., 1998; Garcia *et al*., 2000; Gauquelin *et al*., 2002). This aspect should be explored in future investigations.

Conclusion

Results of cone and seed morphological traits show there is much character similarity among different populations. However, given the morphological diversity among different populations of *Juniperus excelsa* in Balochistan it is concluded that there has been long time held effective geographical segregation among the Juniper tracts of Balochistan, Pakistan. For a better interpretation of the morphological variation and particularly character similarity between distantly located Ziarat and Harboi provenances, the morphological data should be correlated with phylogenetic relationship of populations at molecular level and biogeographic studies. Seed collection should be carried out from the medium altitude for the studies aimed at regeneration of the species as the marginal populations have been found to produce inviable and low quality seed.

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References

Adams RP, Demeke T, Abulfatih HA. 1993. RAPD DNA fingerprints and terpenoids: Clues to past migrations of *Juniperus* in Arabia and east Africa. Theoretical and Applied Genetics **87,** 22-26.

Adams RP, Demeke T. 1993. Systematic relationships in *Juniperus* based on random amplified polymorphic DNAs (RAPDs). Taxon **42,** 553-571.

Adams RP. 2008. Junipers of the world: The genus *Juniperus*, 4th Edn. Bloomington Indianapolis, USA: Trafford Publishing Company Ltd.

Ahani H, Jalilvand H, Nasr HMS, Kouhbanani SH, Ghazi RM, Mohammadzadeh H. 2013. Reproduction of Juniper (*Juniperus polycarpos*) in KhorasanRazavi, Iran. Forest Science Practice **15(3),** 231-237.

Ahmed M, Naqvi EE, Wang LM. 1990. Present state of Juniper in Rodh-Mallazai Forest of Baluchistan, Pakistan. Pakistan Journal Forestry **14,** 227-236.

Arista M, Talavera S. 1996. Density effect on the fruit-set, seed crop viability and seedling vigor of *Abies pinsapo*. Annals of Botany **77,** 187-192.

Barbero M,Lebreton PH,Quezel P.1994. Sur les affinities bio systématiques etphy toécologiques de *Juniperus thurifera* L. et de *Juniperus excelsa* Bieb. Ecologia Mediterranea **20(3/4),** 21-37.

Brus R, Ballian D, Zhelev P, Pandž M, Bobinac M, Acevski J, Raftoyannis Y, Jarni K. 2010. Absence of geographical structure of morphological variation in *Juniperus oxycedrus* L. subsp. *oxycedrus* in the Balkan Peninsula. European Journal of Forest Research **10,** 1007/s10342-010-0457-1.

Champion HG, Seth SK, Khattak GM. 1965. Forests types of Pakistan. Peshawar, Pakistan: Pakistan Forest Institute Press.

Cruden RW. 1972. Pollinators at high elevations ecosystems: Relative effectiveness of birds and bees. Science **176,** 1439-1440.

Douaihy B, Sobierajska K, Jasinska AK, Boratynska K, Tolga OK, Romo A, Machon N, Didukh Y, Dagher-Kharrat MB, Boratynski A. 2012. Morphological versus molecular markers to describe variability in *Juniperus excelsa* subsp. *excelsa* (Cupressaceae). AoB Plants.
Doi: 10.1093/ aobpla/pls013.

Douaihy B, Vendramin GG, Boratyn´ski A, Machon N, Bou Dagher-Kharrat M. 2011. High genetic diversity with moderate differentiation in *Juniperus excelsa* from Lebanon and the eastern Mediterranean region. AoB Plants.
Doi: **10,**1093/ aobpla/plr003.

Farjon A. 1992.The taxonomy of multiseed Junipers (*Juniperus* sect. Sabina) in South west Asia and East Africa (Taxonomic notes on Cupressaceae). Edinburgh Journal of Botany **49,** 251-283.

Farjon A. 1998. World checklist and bibliography of conifers, Kew UK: Royal Botanic Gardens.

Farjon A. 2005. A monograph of Cupressaceae and Sciadopitys. Richmond, Surrey: Royal Botanic Gardens, Kew.

Farjon A. 2010. A handbook of the world's conifers, Vol. II. Leiden, Nederland: Brill Academic Publishers.

Fuentes M, Schupp EW. 1998. Empty seeds reduce seed predation by birds in *Juniperus osteosperma*. Evolutionary Ecology **12,** 823-827.

Garcia D, Zamora R, Gómez JM, Jordano P, Hódar JA. 2000. Geographical variation in seed production, predation and abortion in *Juniperus communis* throughout its range in Europe. Journal of Ecology **88,** 436-446.

Gauquelin T, Bertaudière-Montès V, Badri W, Montès N. 2002. Sex ratio and sexual dimorphism in mountain dioeciousthuriferous Juniper (*Juniperus thurifera* L. Cupressaceae). Botanical Journal of the Linnean Society **138,** 237-244.

Houle G, Babeux P. 1994. Variations in rooting ability and in seed characteristics of five populations of *Juniperus communis* var. *depressa* from subarctic Quebec. Canadian Journal of Botany **72**, 493-498.

Huntley B, Birks HB. 1983. An atlas of past and present pollen maps for Europe, 0-13000 years ago. Cambridge UK: Cambridge University Press.

ISTA. (International Seed Testing Association). 1996. International rules for seed testing. Rules and annexes 1993. Seed Science and Technology **21,** 1-288.

Jimenez JF, Werner O, Sanchez-Gomez P, Fernandez S, Guerra J. 2003. Genetic variation and migration pathway of *Juniperus thurifera* L. (Cupressaceae) in the western Mediterranean region. Israel Journal of Plant Science **51,** 11-22.

Kaplan DR. 2001. The science of plant morphology: definition, history, and role in modern biology. American Journal of Botany **88,** 1711-1741.

Khajjak MH, Raza AM, Shawani MN, Fayyaz A, Ghazala S, Saeed M. 2012. Comparative analysis of essential oil contents of *Juniperus excelsa* (M. Bieb.) found in Balochistan, Pakistan. African Journal of Biotechnology **11,** 8154-8159.

Klimko M, Boratynska K, Montserrat JMY, Didukh Y, Romo A, Gomez D, Kluza-wieloch M, Marcysiak K, Boratynski A. 2007.Morphological variation of *Juniperus oxycedrus* subsp. *Oxycedrus* (Cupressaceae) in the Mediterranean region. Flora **202,** 133-147.

Marcysiak K, Mazur M, Romo A, Montserrat JM, Didukh Y, Boratynska K, Jasinska A,Kosinski P, Boratynski A. 2007. Numerical taxonomy of *Juniperus thurifera*, *J. excelsa* and *J. foetidissima* (Cupressaceae) based on morphological characters. Botanical Journal of the Linnean Society **155,** 483-495.

Mazur M, Boratynska K, Marcysiak K, Didukh Y, Romo A, Kosinski P, Boratynski A. 2004. Low level of inter-populational differentiation in *Juniperus excelsa* M. Bieb. (Cupressaceae). Dendrobiology **52,** 39-46.

Mazur M, Klajbor K, Kielich M, Sowińska M, Romo A, Montserrat JM, Boratyński A. 2010. Intra-specific differentiation of *Juniperus phoenicea* in the western Mediterranean region revealed in morphological multivariate analysis. Dendrobiology **63,** 21-23.

Mohan RB, Uma SB, Ganeshaiah KN. 1996 Intra-fruit seed abortion in a wing dispersed tree, *Dalbergia sissoo* Roxb: Proximate mechanism. Sexual Plant Reproduction **9,** 273-278.

Moore PD, Webb JA, Collinson ME. 1991. Pollen analysis. Oxford, UK: Blackwell Scientific Publications Ltd. 216p.

Mugnaini S, Nepi M, Guarnieri M, Piotto B, Pacini E. 2007. Pollination drop in *Juniperus communis*: Response to deposited material. Annals of Botany **100,** 1475-1481.

Nasir E, Siddiqui MA, Ali Z. 1960. Gymnosperms of West Pakistan. Rawalpindi Pakistan: Gordon College.

Ortiz PL, Arista M, Talavera S. 1998. Low reproductive success in two subspecies of *Juniperus oxycedrus* L. International Journal of Plant Science **159,** 843-847.

Ortiz PL, Arista M, Talavera S. 2002. Sex ratio and reproductive effort in the dioecious *Juniperus communis* subsp. Alpina (Suter) Čelak. (Cupressaceae) along an altitudinal gradient. Annals of Botany **89,** 205-211.

Rafi M. 1965. Vegetation types of Balochistan Province. Lahore Pakistan: Pakistan Government Printing Press.

Sarangzai AM, Moinuddin A, Alia A, Lubna T, Umer JS. 2012. The ecology and dynamics of *Juniperus excelsa* forest in Balochistan-Pakistan. Pakistan Journal Botany **44(5),** 1617-1625.

Schulz C, Jagel A, Stützel T. 2003. Cone morphology in *Juniperus* in the light of cone evolution in Cupressaceae. Flora **198,** 161-177.

Shaheen H, Qureshi AR, Shinwari Z. 2011. Structural diversity, Vegetation dynamics and anthropogenic impact on lesser Himalayan subtropical forests of Bagh district, Kashmir. Pakistan Journal Botany **43(4),** 1861-1866.

Sheikh IS. 1985. Afforestation in Juniper forests of Balochistan. Peshawar Pakistan: Pakistan Forest Institute.

Sultangaziev O, Schueler S, Geburek T. 2010. Morphometric traits and sexual dimorphisms do not strongly differentiate populations of Zeravshan Juniper (*Juniperus seravschanica* kom.) in Kyrgystan. Flora **205,** 532-539.

Takhtajan Al. 1986. Floristic regions of the world. Berkeley USA: University of California Press.

Ward LK. 2007. Lifetime sexual dimorphism in *Juniperus communis* var. *communis*. Plant Species Biology **22,** 11-12.

Willson MF, Burley N. 1983. Mate choice in plants. Princeton NJ USA: University Press, Princeton.