Pak. J. Bot., 44(6): 1977-1982, 2012.

CYTOLOGICAL STUDIES ON 14 PLANT SPECIES UNDER POLLUTED CONDITIONS

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Abstract

Thirty four specimens of 14 species belonging to Cyperaceae and Poaceae growing in the vicinity of industries, agricultural fields and a combination of both were collected in and around Karachi. The study aimed to find out how the plants are affected by a long-term exposure to a number of pollutants focusing on the meiotic behavior (precocious chromosomes, chromosomal stickening, split spindles, lagging chromosomes), dyads formation and pollen stertility. The percentage of meiotic abnormalities in the specimens from polluted sites was significantly higher as compared to their respective controls. The specimens from polluted localities showed a greater tendency to produce dyads as compared to controls. Voucher specimens of 9 species produced significant number of sterile pollens under polluted condition.

Introduction

Like other developing countries, in Pakistan the ecosystems are also exposed to one or other types of pollutants that are disturbing the natural biodiversity in many ways. Rapid urbanization and industrialization have aggravated the problem manifold in last couple of years. Karachi is a hub of trade and industry with a dense human population in its urban areas whereas in the sub-urban parts there are fairly large agricultural areas in Gadap and Malir. Two seasonal rivers Lyari and Malir traverse the Karachi division; however the unchecked release of untreated industrial and municipal waste water over the decades has converted them into effluent drain. About 80% of the wastewater of Karachi is discharged into the sea through these rivers (Blumer *et al.,* 1973). Pollution in the neighbor hood of the coastal cities is becoming a problem all over the world but Karachi coastal area has suffered more seriously than any other part of the globe (Khan *et al.,* 1999). The industrial effluents along with sewerage water are contaminating the vegetables being grown in the area and increasing the metal content in the ground water underneath. Besides industrial pollution, agriculture is another major source of pollution. Due to heavy usage of pesticides and fertilizers associated with the modern agriculture, effluents from agricultural lands contain pesticide residue and excessive fertilizers, which can pollute the natural surface and ground water resources. In addition to polluted soil and water; the atmospheric pollution in Karachi is also quite high. The air and water pollution limits in Karachi have crossed national and international environmental quality standards posing serious threats to the lives of 18 to 20 million residents (Anon., 2006).

Crop and wild plants are some of the frequent recipients of pesticides among biota (Hardy 1982, Costa *et al.,* 1987). This is why plants can be the best detectors of all kinds of hazardous pollutants and can be used to remove or destroy hazardous contaminants from media such as air, water and soil called phytoremediation (Terry & Bañuelos, 2000). This relevant problem stimulates interest in investigating toxicological, carcinogenic and mutagenic effects of industrial chemicals (Ta'trai *et al.,* 1980). Different types of mitotic and meiotic abnormalities in *Vicia faba* were observed when exposed to Cadmium and Lead. Yasmin *et al.,* (2o11) in their study concluded that the effluents coming from textile industries effect the growth of *Lens esculentum* more severely as compared to marble industry and oil refinery. Kakar *et al.,* (2010) studied the effect of waste water on growth and development of canola. They observed that plants which received polluted water were weak, have less height, and reduced fresh & dry shoot and root weights. Kabir *et al.,* (2010) in their study concluded that industrial wastes make the soil polluted due to which a negative impact was observed on the ecosystem. Feretti *et al.,* (2007) reported the presence of 33 pesticides (including some unapproved ones) from a sample of 21 vegetables and 8 types of grapes in southern Italy with much higher genotoxicity.

Most of the work on the effects of pollution on plants has been conducted on cultivated plants particularly the crop plants. Very little attention has been given to the effects of pollution on plants in the natural and semi natural ecosystem. Therefore the aim of this study is to find out the possible genotoxic effects of pollutants (agricultural, industrial and both) in wild plant species exposed for many generations.

Material and Methods

Plant material was collected from the natural plant populations at various localities exposed to agricultural, industrial and mixed pollutants in Karachi and near-by places. The collection sites included 1) Behind Agriuto Ind. Hub, (Dist. Lasbella), 2) Inside Baluchistan Lubricants Ltd. Hub (Dist. Lasbella), 3) Agr. Field near Bhambore museum turning (Dist. Thatta), 4) Tomato Field 15 Km from Gadap Town. (Dist. Dadu), 5) Behind Gatron Industries, Main RCD highway (District Lasbella), 6) Gharo Sem Nala (Dist. Thatta), 7) Gujjo (Dist. Thatta), 8) Korangi kreek (Karachi Division), 9) Korangi Road (Karachi Division), 10) Lyari river (Karachi Division), 11) Malir River (Karachi Division), 12) Nooriabad Industrial Area (Dist. Dadu) and 13) Shah Faisal Colony. (Karachi Division) The specimens of same plant species were collected from localities with no apparent source of pollution as controls.

For cytological studies young, mature and fully grown floral buds were fixed in Carnoy's solution (Ethanol & Glacial Acetic Acid, 3:1) at the spot in air tight vials and stored in refrigerator till the preparation of slides. Slides were prepared from young, mature and fully grown anthers by squash technique with 1 % propionic carmine to observe meiotic abnormalities, dyad formation and sterile pollen grains respectively.

For meiotic abnormalities, at every stage 20-100 cells were observed depending upon their availability. Meiotic product *i.e.* tetrads and dyads frequencies were also observed by counting 100 or more meiotic products. For pollen fertility test, mature pollens were squashed in 1% Propionic acid and left for 15 minutes for staining. On average 100 or more pollen grains were counted. The dark colored pollen grains were scored as fertile while the light and colorless were considered as sterile. Good contrast pollen mother cells (PMC's) with meiotic abnormalities were photographed by Nikon photomicroscope. Voucher specimens have been deposited in Karachi University Herbarium (KUH).

Z-Test (Zar, 1996) was performed to check the significant statistical differences between control and test plants.

Results and Discussion

The results clearly show that the percentage of PMC's with meiotic abnormalities in the plants growing in polluted areas (industrial, agricultural and combination of both) is distinctly higher as compared to the control specimens, almost in all the 14 species. Higher percentages of meiotic abnormalities were observed in meiosis I as compared to meiosis II stage and most of the abnormalities have been observed at metaphase-I, II and anaphase-I, II stages of meiosis. The most frequent abnormalities were precocious chromosomes at metaphase I and II and lagging chromosome at anaphase I and II whereas stickiness and split spindle formation was also observed in some cells (Fig. 1, a-i).

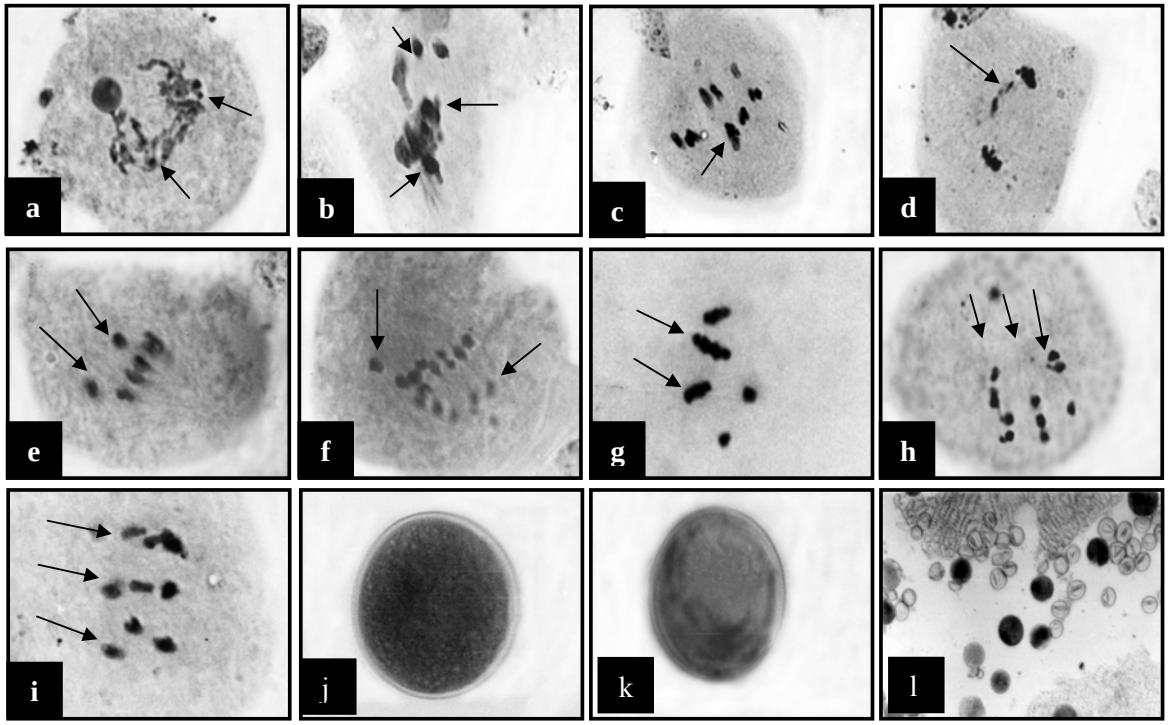

Fig. 1. a. *Aeluropus lagopoides* SR 226 (X1000) Disturbed Diakinesis with stickiness. b. *Cenchrus ciliaris* SR 493 (X1000) Precocious bivalents at Metaphase-1, c,d. *Cenchrus pennisetiformis* SR 174 (X1000), c. Precocious bivalents at Metaphase-1. d. Lagging chromosomes at Anaphase-1. e, f. *Cynodon dactylon* SR 413 (X1000) Metaphase-1 with precocious chromosomes. g-i. *Paspalidium geminatum* SR 67 (X1000), g. multivalent at metaphase-1, h, i. Precocious bivalents at metaphase-1with split spindle, j-l. *Echinochloa colona* SR 257, j. Diploid fertile pollen grain (X1000), k. Diploid sterile pollen grain (X1000), l. Diploid fertlile, diplod sterile, haploid fertile and haploid sterile pollen grains (X400).

The most prominent species which showed highest percentages of overall meiotic abnormalities are *Cyperus arenarius* (SR 91) (98.61%), *Cyperus laevigatus* (SR 65) (88.82%), *Cyperus rotundus* (SR 527) (76.38%) and *Paspalidium geminatum* (SR 67) (75%). Stage-wise maximum meiotic abnormality at Metaphase-I was observed in *Cyperus arenarius*, (SR 91) i.e., 97.22, at Metahase-II in *Paspalidium geminatum* (SR 66) i.e., 82.5%, in Anaphase-I *Cyperus arenarius* (SR 91) i.e., 100% and in Anaphase-II *Cenchrus ciliaris* (SR 493) i.e., 66.66% .Results of meiotic abnormalities are summarized in Tables 1, 3 and Fig. 2.

Z-Test also resulted significantly in most of the species examined ($p<0.001$***, Table 2) whereas only in two cases i.e., *Eragrostris ciliaris* (SR 556) and *Ochtochloa compressa* (SR 566) level of significance is observed to be $p<0.01$** and $p<0.05$*, respectively (Table 2). Amer & Farah (1983 a, b) reported same kind of meiotic abnormalities in *Vicia faba* when treated with few pesticides. They also reported the reduction in percentage of meiotic abnormalities in meiosis II than I. A comparison of total meiotic abnormalities in test and control specimens show that the majority of control plants have meiotic abnormalities within the range of 1-20% while the maximum meiotic abnormalities in plants exposed to pollutants range between 50-70% (Fig. 3).

Table 1. Percentages of meiotic abnormalities in plants exposed to industrial, agricultural and mixed pollutants with respect to their controls.

S. #	Species with family name	Voucher #	Cells with meiotic abnormalities (%)				
			M I	M II	A I	A II	Total Ab.
	Cyperaceae						
1.	*Bolboschoenus glaucus* (Lam.) S.G. Smith.	SR 821	47.82	…	27.27	…	41.17
		SR 833 C	5.34	1.25	2.36	0	2.77
2.	*Cyperus arenarius* Retz.	SR 91	97.22	….	100	….	98.61
		SR 852 C	25	19.35	20	0	21.98
3.	*Cyperus laevigatus* L.	SR 65	87.2	…	85.71	…	88.82
		SR 72	86.06	…	….	…	86.06
		SR 849 C	14.06	6.25	19.56	60	26.31
4.	*Cyperus rotundus* L.	SR 153	65.12	61.01	81.21	…	71.24
		SR 527	93.14	41.25	84.61	51.14	76.38
		SZ 209 C	1.98	3.85	…	…	2.1
	Poaceae						
5.	*Aeluropus lagopoides* (L.) Trin. ex Thw.	SR 223	91.25	64.66	54.32	…	73.65
		SR 226	81.98	67.25	51.26	43.25	71.26
		SR 292 C	12.63	…	6.23	…	10.34
6.	*Cenchrus ciliaris* L.	SR 493	92.59	76.92	45.69	66.66	70.46
		SR 790 C	21.36	9.42	19.11	…	16.63
7.	*Cenchrus pennisetiformis* Hochst. & Steud.	SR 174	82.56	48.97	86.36	…	76.56
		SZ 876 C	8.33	0	0	3.7	2.88
8.	*Cynodon dactylon* (L.) Pers.	SR 413	50	24	21.05	…	36.66
		SR 552 C	21.36	9.42	19.11	…	16.63
9.	*Desmostachya bipinnata* (L.) Stapf	SR 237	23.07	…	59.37	…	48.88
		SR 846 C	13.04	4.76	22.02	0	12.3
10.	*Eragrostris ciliaris* (L.) R. Br.	SR 556	41.66	…	27.27	…	37.14
		SR 777 C	26.08	3.12	33	0	11.33
11.	*Ochtochloa compressa* (Forsk.) Hilu.	SR 566	11.1	…	4	…	7.4
		SR 816 C	1.96	2.44	0.92	1.28	1.65
12.	*Panicum turgidum* Forsk.	SR 71	51.61	25.02	19.54	42	42.03
		SZ 852 C	13.8	0	4.35	0	5.02
13.	*Paspalidium geminatum* (Forsk.) Stapf	SR 66	84.8	82.5	52.78	…	43.36
		SR 67	67	…	85	..	75
		SR 257	55	…	42.5	…	40
		SR 717	56.52	…	…	…	56.52
		SR 113 C	12	6	22	8.04	10.63
14.	*Tragus roxburghii* Panigrahi	SR 732	17.92	…	47.82	…	29.03
		Kh 1742 C	3.77	…	2.43	…	3.19

Note: A1= Anaphase 1, AII = Anaphase II, MI= Metaphase I, MII= Metaphase II, T. Ab= Total Abnormality, SR= Sadaf Rahimi, Kh.= Khatoon, C= Control

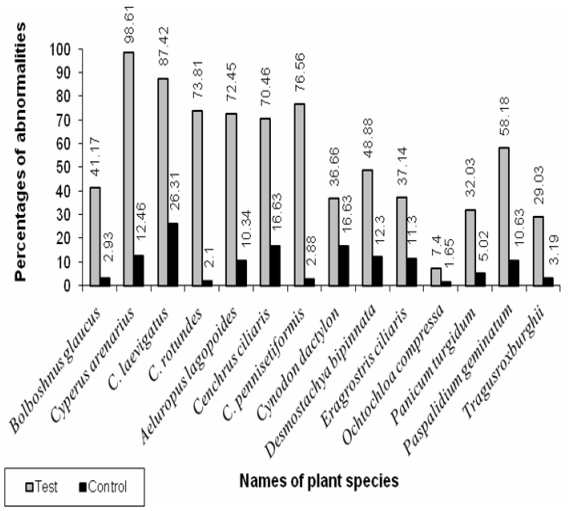

Fig. 2. Comparison of meiotic abnormalities between the members of Poaceae and Cyperaceae with respect to their controls.

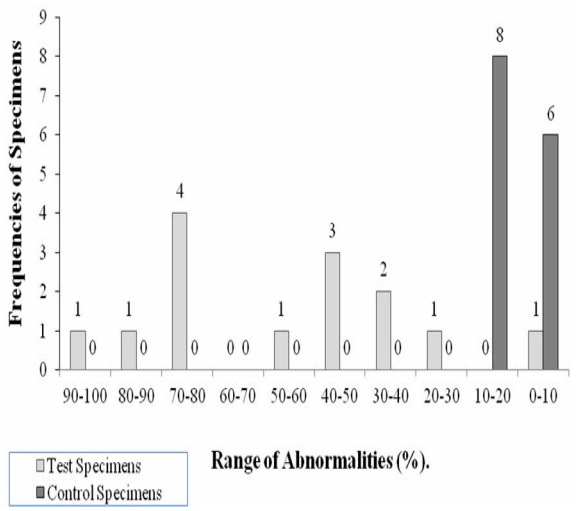

Fig. 3. Comparison of frequencies of meiotic abnormalities in test and control specimens.

Table 2. Statistical comparison of meiotic abnormalities in plants exposed to industrial, agricultural and mixed pollutants with respect to their controls.

S. #	Species with family name	Voucher #	Z-Test value	Level of significance
	Cyperaceae			
1.	*Bolboschoenus glaucus* (Lam.) S.G. Smith.	SR 821	9.6	0.001***
2.	*Cyperus arenarius* Retz.	SR 91	25.5	0.001***
3.	*Cyperus laevigatus* L.	SR 65	15.6	0.001***
		SR 72	14.9	0.001***
4.	*Cyperus rotundus* L.	SR 153	13.8	0.001***
		SR 527	33.78	0.001***
	Poaceae			
5.	*Aeluropus lagopoides* (L.) Trin. ex Thw.	SR 223	12.33	0.001***
		SR 226	10.6	0.001***
6.	*Cenchrus ciliaris* L.	SR 493	10.78	0.001***
7.	*Cenchrus pennisetiformis* Hochst. & Steud.	SR 174	18.42	0.001***
8.	*Cynodon dactylon* (L.) Pers.	SR 413	5	0.001***
9.	*Desmostachya bipinnata* (L.) Stapf	SR 237	5.2	0.001***
10.	*Eragrostris ciliaris* (L.) R. Br.	SR 556	3.2	0.01**
11.	*Ochtochloa compressa* (Forsk.) Hilu.	SR 566	1.91	0.05*
12.	*Panicum turgidum* Forsk.	SR 71	5.1	0.001***
13.	*Paspalidium geminatum* (Forsk.) Stapf	SR 66	36.5	0.001***
		SR 67	16.25	0.001***
		SR 257	5.04	0.001***
		SR 717	9.14	0.001***
14.	*Tragus roxburghii* Panigrahi	SR 732	4.3	0.001***

Table 3. Percentages of dyads and pollen sterilities in plants exposed to industrial, agricultural and mixed pollutants with respect to their controls.

S #	Species with family name	Voucher specimen #	Dyads (%)	Sterile pollens (%)
	Cyperaceae			
1.	*Bolboschoenus glaucus* (Lam.) S.G. Smith.	SR 821	0	1.91
		SR 833 C	0.96	0
2.	*Cyperus arenarius* Retz.	SR 91	….	13.99
		SR 549 C	2.56	0
3.	*Cyperus laevigatus* L.	SR 65	….	8.21
		SR 72	….	….
		SR 849 C		4.28
4.	*Cyperus rotundus* L.	SR 153	0	7.6
		SR 527	0	0
		SZ 209 C	0	0.5
	Poaceae			
5.	*Aeluropus lagopoides* (L.) Trin. ex Thw.	SR 223	2.74	1.9
		SR 226	6.3	9.32
		SR 292 C	0.69	0
6.	*Cenchrus ciliaris* L.	SR 493	10.88	13.85
		SR 790 C	0	4.05
7.	*Cenchrus pennisetiformis* Hochst. & Steud.	SR 174	18.54	35.08
		SZ 876 C		
8.	*Cynodon dactylon* (L.) Pers.	SR 413	20.1	16.35
		SR 552 C	….	9.53
9.	*Desmostachya bipinnata* (L.) Stapf	SR 237	….	12.12
		SR 846 C	…..	2.36
10.	*Eragrostris ciliaris* (L.) R. Br.	SR 556	….	5.88
		SR 777 C	…	2.43
11.	*Ochtochloa compressa* (Forsk.) Hilu.	SR 566	0	2.44
		SR 816 C	0	0
12.	*Panicum turgidum* Forsk.	SR 71	4.21	6.12
		SZ 852 C	0	0
13.	*Paspalidium geminatum* (Forsk.) Stapf	SR 66	….	6
		SR 67	….	8.02
		SR 257	49.01	55.08
		SR 717	2.66	7.84
		SR 113 C	2	3.38
14.	*Tragus roxburghii* Panigrahi	SR 732	….	0
		KH 1742 C	….	0

Table 4. Statistical comparison of pollen sterilities in plants exposed to Industrial, agricultural and mixed pollutants with respect to their controls.

S #	Species with family name	Voucher specimen #	Z-Test value	Level of significance
	Cyperaceae			
1.	*Bolboschoenus glaucus* (Lam.) S.G. Smith.	SR 821	2.48	0.05*
2.	*Cyperus arenarius* Retz.	SR 91	7	0.001***
3.	*Cyperus laevigatus* L.	SR 65	1.87	N.S.
4.	*Cyperus rotundus* L.	SR 153	8.57	0.001***
	Poaceae			
5.	*Aeluropus lagopoides* (L.) Trin. ex Thw.	SR 223	2	0.05*
		SR 226	5.14	0.001***
6.	*Cenchrus ciliaris* L.	SR 493	2.41	0.05*
7.	*Cenchrus pennisetiformis* Hochst. & Steud.	SR 174	5.55	0.001***
8.	*Cynodon dactylon* (L.) Pers.	SR 413	3.22	0.01**
9.	*Desmostachya bipinnata* (L.) Stapf	SR 237	0.09	N.S.
10.	*Eragrostris ciliaris* (L.) R. Br.	SR 556	0.93	N.S.
11.	*Ochtochloa compressa* (Forsk.) Hilu.	SR 566	1.43	N.S.
12.	*Panicum turgidum* Forsk.	SR 71	3.11	0.01**
13.	*Paspalidium geminatum* (Forsk.) Stapf	SR 66	1.02	N.S.
		SR 67	2.62	0.05*
		SR 257	17.8	0.001***
		SR 717	1.49	N.S.

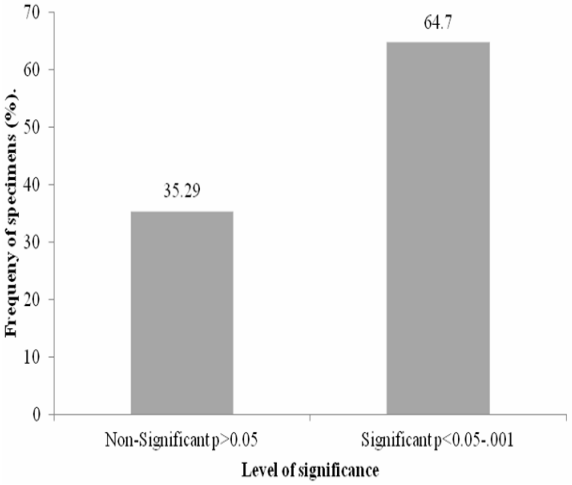

Fig. 4. Comparison of significant and non-significant cases in pollen sterility of test and control plants based on Z-test.

Besides the abnormalities in various stages of meiosis, the specimens from polluted localities have also showed certain percentages of dyads as the meiotic product in addition to normal microspore tetrads (Table 3). Statistical analysis of meiotic product and pollen sterility also revealed significant differences in most of the cases (Table 4). The highest percentage of dyad was found in *Paspalidium geminatum (*SR 257) (49.01%) followed by *Cenchrus pennisetiformis* (SR 174) (18.54%) and *Cenchrus ciliaris* (SR 493) (10.88%). These specimens showed higher percentages of pollen sterility in their pollen fertility test. Pollen sterility is also evident from *Echinocloa colona* (SR 257) (Fig. 1j-l). Z-Test of pollen sterility also yielded significant differences in 64.7% cases whereas 35.29% non significant (Fig. 4).

The above observations clearly descant that non-point source pollutants increase the frequency of meiotic abnormalities and enhance the production of diploid and sterile pollen grains. If the pollen grains with meiotic abnormalities fertilize an ovule, the abnormalities would pass to the next generation and if diploid pollen grains fertilize the ovule, the offspring would be a polyploid. Grant & Zura (1982) reported that pesticides which induce chromosomal abnormalities also reduce pollen viability.

Conclusions

The present study shows that pollution emanating from many sources adversely affects the reproductive biology of plant species in natural and semi-natural ecosystems particularly at chromosomal level causing meiotic abnormalities with possible mutagenic effects. The meiotic disturbances can induce pollen sterility and formation of diploid gametes that would ultimately lead to polyploidy, which is a threat to our biodiversity.

Acknowledgement

Deep gratitude is due to Prof. Dr. Shahid Shaukat, Department of Environmental Science, University of Karachi for his help in statistical analysis. I am extremely thankful to Mr. M. Imran for help and assistance in lab work and in field collection.

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(Received for publication 25 March 2011)