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Seeds characters, pollen fertility and flavonoids of ten Brassicaceae collected near a kilns thermal power plant for air pollution bioindication

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Abstract. *Noori M, Baghaeifar Z, Chehregani A, Faraki F. 2018. Seeds characters, pollen fertility and flavonoids of ten Brassicaceae collected near a kilns thermal power plant for air pollution bioindication. Nusantara Bioscience 10: 96-104.* Shazand Steam Power Plant located on North-East of Shazand, Iran began to work from 2000. The power plant necessity fuel is natural gas and mostly heavy fuel oil. The most pollutant of power plant is sulfur compounds in addition to nitric and carbon mono oxide. Because environmental pollutants influence plant fertility and chemical compounds, therefore this study was done on ten wild Brassicaceae (*Alyssum linifolium* var. *linifolium, Alyssum longistylum*, *Alyssum marginatum*, *Choriospora persica*, *Clypeola lappacea, Conringia perfoliata*, *Descurainia sophia, Goldbachia laevigata*, *Isatis kotschyana* and *Neslia apiculata*) taxa collected from the thermal power plant area for bioindication of regional air pollution comparing to controls collected 40 km away from the power plant. Brassicaceae members are important for their ecological, pastoral, medicinal and edible points. Seed width and length max. and their ratio and abnormal seed percentage were calculated. Pollen abnormality and sterility percentages determined using Muntezing's acetocarmine and light microscopy. Also, their pollen flavonoids were semi-quantitatively assessed using two-dimensional paper and thin layer chromatography. Results showed seeds health and their dimensions reduction in polluted samples in comparison with controls. In *C. lappacea* significant differences of seed and pollen abnormality and pollen sterility percentages, morin and kaempferol concentrations, between control and polluted samples were observed ($P \leq 0.05$). Also, number and kind of pollen flavonoid changes especially increasing flavonoid contents were observed in polluted plants comparing to control. Studying seed and pollen characters can be used as air quality bioindicators.

Keywords: Air pollution, bioindicator, Brassicaceae, flavonoids, pollen, power plant

INTRODUCTION

Thermal power plants are designed in a variety of fashions depending on the available fuel, mostly natural gas and heavy fuel oil. Depending on the fired fuel, emission of thermal power plants is sulphuric and nitric oxides. Sulfur oxides constitute the most common pollution when burning heavy fuel oil (Noori et al. 2014). By the reason Brassicaceae taxa importance from ecological, pastoral, medicinal and edible points, this study was done on 10 collected Brassicaceae taxa from the area and their controls.

Using plants for biomonitoring of air and soil quality may turn out to be successful, as they are simple, cheap and fast. They can supplement the classical physicochemical methods. By these types of techniques, the information on the pollutants can be derived from the study of the biological responses of plants to pollution (Malayeri et al. 2007). Many authors studied on reactions of plants to air pollutants, and those species suitable as plant bioindicators (Gottardini et al. 2004). Bosac et al. (1994) investigated the impact of O_3 and SO_2 on reproductive development of oil seed rape (*Brassica napus*), *pollen* germination. Pollen as a biologic allergen is a sensitive bioindicator of atmospheric pollution and provides particularly original and interesting information on the potential adverse effects of pollutants on living organisms (Yosefi et al. 2011). Air pollution affects on growth, development, morphobiometrical and phytochemical pollen grains parameters. Many pollutants have direct impact on the pollen physiology; and indirect impact on its ontogenesis via their effects on plant reproduction. It may be pointed out that this ontogenesis is also subordinated to the other environmental factors (atmospheric and/or edaphic) acting on the producing plants. Of course, pollen does not indicate levels of pollutants, but it measures their biological impact (Gottardini et al. 2004).

Pollen viability and morphology were used for fluoride pollution biomonitoring (Malayeri et al. 2011). Studies on collected pollen from polluted and unpolluted areas have shown contradictory results. Acid rain could affect developmental process of pollen grains. Exine was not formed in some case of plants that treated by different acid solutions (Chehregany et al. 2006). Pollination with SO_2 fumigated pollen resulted in reduced seed production and weight (Elke 1990). Low-level O_3 and/or SO_2 exposure causes a linear decline in soybean yield and high-level exposure caused significant linear reductions of 4-7% in mass per seed, seed number and weight (Sprugel et al. 1980, Reich and Amundson 1984). Studies on extracts of exposed *Quercus rubra*, *Festuca elatior* and *Ulmas pumila* pollen grains with CO, SO_2 and NO_2 using two-dimensional thin layer chromatography and SDS-gel electrophoresis (PAGE) methods showed changing amino

acids and molecular weight profiles. Also, antigenic changes were observed in contaminated pollen comparing to control using double immune-diffusion method (Ruffin et al. 1986). Air pollution effects on structures, proteins, and allergenicity of pollen grains using SDS-PAGE method showed decreasing protein content in all of polluted samples in response to air pollution. (Majd et al. 2004). Changing pollen protein profiles and appearing some new bands in DEP (diesel exhaust particles)-exposed pollen grains were observed in polluted *Lilium martagon* pollen extract comparing to control (Chehregany and Kouhkan 2008).

Anthocyanins were as an indicator for plant responses to environmental stress (Chalker-Scot 1999). Polyphenolics were introduced as indicator to different environmental stresses. It shows plant metabolic adaptation with increasing flavonoids content responding to environmental pollution (Rezanezhad and Nasibi 2006). Rezanejad (2012) stated that phenolic compounds function as stress indicators because they accumulate to high levels in many plant tissues in response to a wide range of biotic and abiotic signals. These compounds are involved in pollen development, pollination, pollen germination, and pollen tube growth (Rezanejad 2012). Feucht and Treutter (1999) studied the role of flavan-3-ols in plant defense. They found that flavan-3-ols and other phenolic compounds as unsaturated oil acids protective are reported against free radicals. Also flavanols increase cell membrane protective agents (Feucht and Treutter 1999). Analyzing polluted pollen grain extracts, using HPLC showed increasing flavonoids level in polluted pollen in comparison with control (Rezanejad 2012). Such studies indicated that seeds and pollen grains provide essential information on biological impact of pollutants and they are good candidates for biomonitoring the atmospheric and edaphic pollutions.

In this study effects of Shazand Thermal Power Plant (Iran) pollution on seeds characters, pollen fertility and flavonoids profile in 10 collected Brassicaceae taxa from the Power Plant area (having SO_2, CO, NO) in comparison with control 40 km away from the power plant (no this pollutant) were investigated. Also about using these characters as air pollution bioindicators is discussed.

MATERIALS AND METHODS

Collection of plant material, determination, and seed morpho-biometry

Plant material of ten wild and native Brassicaceae taxa (*Alyssum linifolium* var. *linifolium*, *Alyssum longistylum*, *Alyssum marginatum*, *Choriospora persica*, *Clypeola lappacea*, *Conringia perfoliata*, *Descurainia sophia*, *Goldbachia laevigata*, *Isatis kotschyana* and *Neslia apiculata*) were collected (five samples of each taxa) from the Shazand Thermal Power Plant (http://www.mapna.com/en/products/power) area located at NE of Shazand, Markazi Province, Iran and its geographical coordinates are 33° 53' 25" North, 49° 9' 43" East (Figure 1) (http://www.maplandia.com/iran/markazi/shazand). Control samples were collected from 40 km distances of the Power Plant. Herbarium vouchers were prepared. Collected plant identified using available authoritative references (Rechinger 1968; Mobayen 1979; Ghahreman 1976-2008) (Figure 2).

Seed morphobiometrical studies were done using a zoom binocular light microscope. Seed length maximum (SLM), seed width maximum (SWM), seed length max/seed width max (SLM/SWM), seed diameter max (SDM), normal seed% (NS%) and abnormal seed% (AS%) measured and calculated for 10 plants of each taxon. Round and having kernel seeds were considered normal versus wrinkled, holed and lacked kernel seeds as abnormal.

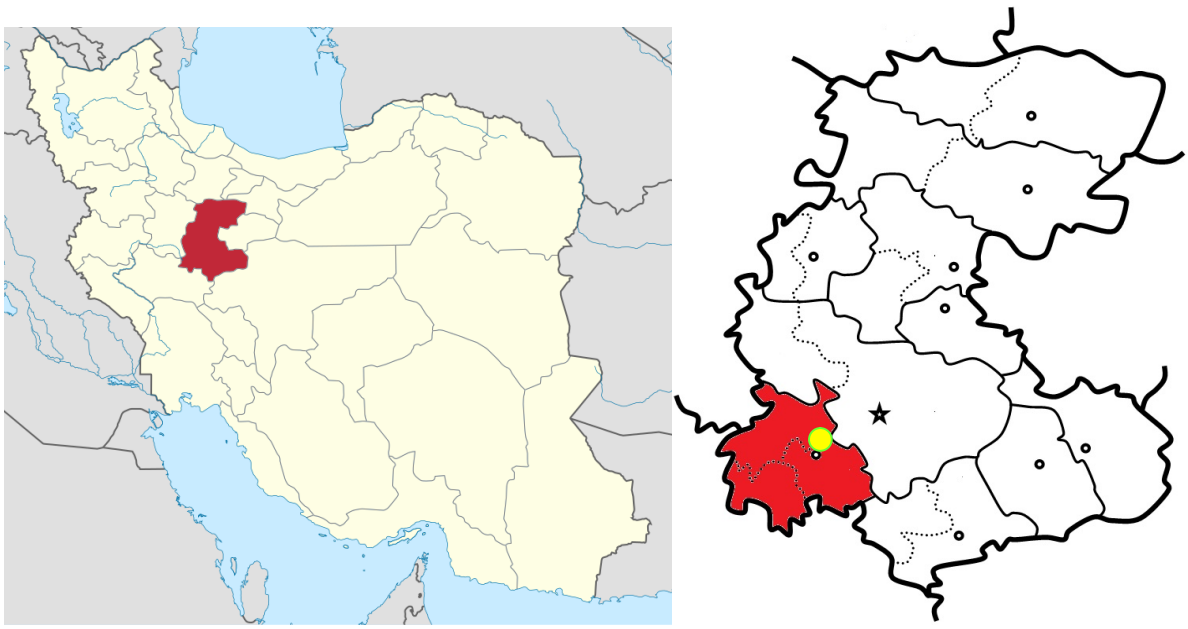

Figure 1. Showing the Shazand Thermal Power Plant area located at NE of Shazand, Markazi Province, Iran

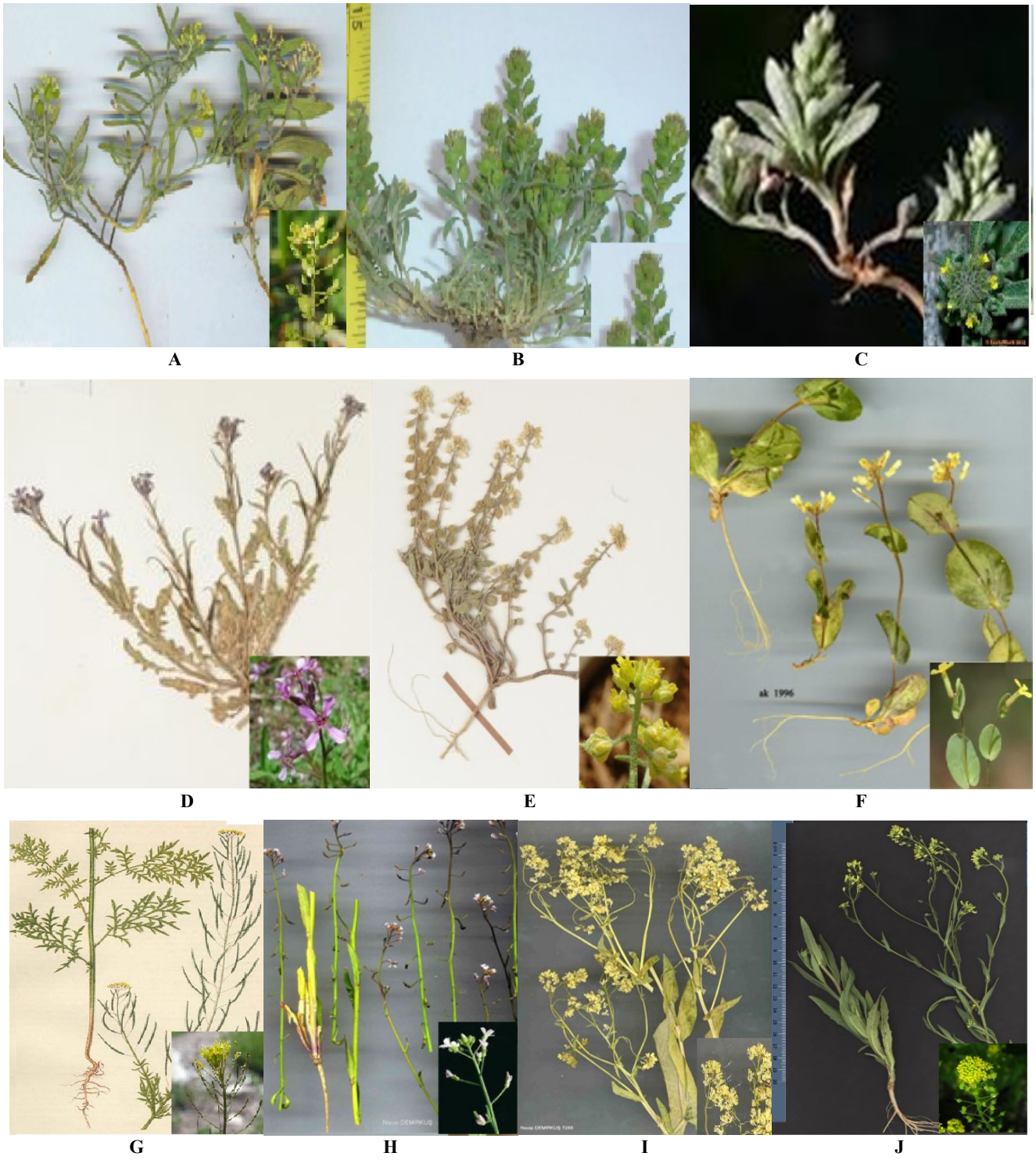

Figure 2. Showing habit of 10 studied Brassicaceae taxa. A. *Alyssum linifolium* var. *linifolium*, B. *Alyssum longstylum*, C. *Alyssum marginatum*, D. *Choriospora persica*, E. *Clypeola lappacea*, F. *Conringia perfoliata*, G. *Descurainia sophia*, H. *Goldbachia laevigata*, I. *Isatis kotschyana*, J. *Neslia apiculata*

Preparation and light microscopy of pollen grains

Flowers of all collected control and polluted samples were fixed in Carnoy's solution (a fixative composed of glacial acetic acid and ethanol 3:1). Pollen grains were studied using Muntezing's acetocarmine method (Noori 2002). Five samples from each species were randomly chosen, and their anthers smashed on a microscopic slide. Empty anther shells were removed, and one drop of the staining solution was applied to the pollen grains on the respective slide. After uniformly dispersing the pollen and stains, they were covered with a cover slip. 24-48 hours later, the slides were observed by light microscopy (Galen III, Leica, Wetzlar, Germany). Palynological characters such as abnormality and sterility percentages were

determined for each control and polluted sample. Pollens were categorized into two groups those with round exine and regular shape (normal) and these with wrinkled, shrunk, fragile exine and irregular shape (abnormal). Percentages of stained pollen (fertile) and non stained pollen (sterile) were also scored.

Preparation and extraction of pollen grains

Pollen grain samples from mature fresh flower anthers were air dried after dissection of the flowers for flavonoids detection and identification. For a comparative analysis of the flavonoids, small extracts of all the accessions were prepared by boiling 200 mg of powdered pollen grain for 2 min in 5 mL 70 % ethanol/water. The mixture was cooled and left to extract for 24 h. The extract was filtered, evaporated to dryness by rotary evaporation at 40°C and taken up in 2 mL 80 % MeOH/water for analysis by two-dimensional paper chromatography (2-D PC) (Noori 2002).

Flavonoid analysis by 2-D PC

Aliquots of 10 times 2 μL of each extract were applied to the corner of a quarter sheet of chromatography paper (Whatman No 1). The chromatograms were developed in the first direction with n-butanol-acetic acid-water=4:1:5 v/v, upper layer (BAW) and in the second direction with 15% aqueous acetic acid, with rutin (= quercetin 3-*O*-rutinoside) as standard. After development, the chromatograms were viewed in UV 366 nm and any dark absorbing and fluorescent spots were marked. R_f -values were calculated (Noori et al. 2002).

Methods of identification of the flavonoids

After obtaining sufficient amounts of purified flavonoids, as in the case of the flavonoids from 10 Brassicaceae taxa pollen, they were identified by means of UV spectroscopy using shift reagents to investigate the substitution patterns of the flavonoids (Mabry et al. 1970; Markham 1982) and by acid hydrolysis to identify the aglycone and sugar moieties. Co-chromatography with standards was also performed where possible. Flavonoid standards available for comparison during the study were apigenin, chrysin, genistein, hesperidin, isorhamnetin, kaempferol, luteolin, morin, myricetin, naringenin, quercetin, rhamnetin, rutin, tricine and vitexin, rutin (ILDIS 1994) from Merck, Darmstadt, Germany; apigenin and luteolin from Sigma, St. Louis, MO, USA; and the others from Fluka-Sigma-Aldrich, Deisenhofen, Germany).

Acid hydrolysis and identification of flavonoid aglycones

After dissolving a small amount of each purified flavonoid (ca 0.5 mg) in 0.5 ml of 80% MeOH in a test tube, 2 ml of 2M HCl were added and the mixture was heated. Then 2 ml of EtOAc were added and thoroughly mixed with the aqueous layer using a Whirley mixer after cooling. The upper EtOAc layer was removed and evaporated to dryness, dissolved in 0.5 ml of MeOH and applied as spots on thin layer chromatograms (cellulose). The TLC plates were run in three solvents alongside standards to identify the aglycone moiety (Harborne 1998). Then TLC chromatograms were viewed in UV 254 nm and each spot R_f -values and color comparing to standards helped to kind of flavonoids identification.

Data analysis

Seed morphobiometrical data results were analyzed by EXCEL. Pollen abnormality and sterility percentages were calculated. Pollen grain flavonoids data were scored. Then all of obtained data were analyzed by ANOVA, Duncan, and Tukey tests ($P \leq 0.05$) using SPSS.

RESULTS AND DISCUSSION

Results

Seed morphobiometrical studies results showed seed dimensions, and seed normality percentage had reduction in all of studied taxa except *Neslia apiculata* species that abnormal seed percentage was higher than in control comparing to polluted (Table 1). Table 2 shows palynological data of polluted samples comparing to control using Muntezing's acetocarmine method. Results showed all of studied control plant pollen grains were elliptic and tricolporate (Figure 3). All of control samples of ten collected Brassicaceae taxa from 10 km distances of the Shazand Thermal Power Plant area exceptional *G. laevigata* and *I. kotschyana* showed 100% fertility in their pollen grains. While, abnormality was observed in all of studied polluted samples. Also their total sterility% increased. Polluted *I. kotschyana* had the most abnormal pollen grains (50.8%) and maximum sterility (45.5%). Minimum abnormality pollen grains (4.9%) and minimum sterility (4.92%) were observed in polluted *G. laevigata*.

Pollen grain flavonoids data of ten studied Brassicaceae taxa from the Shazand Thermal Power Plant area, Iran comparing to control using 2-DPC and TLC methods have been shown in Table 3. As the Table shows flavonoid sulfates and flavon *C*-and *C*- /*O*-glucosides were observed in all of studied control and polluted samples. Aglycones were not found in control and polluted samples of *Alyssum longstylum*, *G. laevigata* and *N. apiculata* species while both control and polluted samples of *A. marginatum* and *C. lappacea* species lacked. Polluted samples of *C. persica*, *D. sophia* and *C. perfoliata* had aglycones comparing to control but *I. kotschyana* and *A. linifolium* var. *linifolium* taxa control samples had aglycones in comparison with polluted. This study revealed high level changing in kind and number of pollen grain flavonoids between control and polluted samples. Flavonoids number increased in polluted samples comparing to control with the exception of *A. marginatum* and *G. laevigata* species. Polluted *N. apiculata* and *A. marginatum* showed the most increasing total flavonoids and *C. lappacea* was at least. Fourteen flavonoids compounds consisting of apigenin, chrysin, genistein, hesperidin, isorhamnetin, kaempferol, luteolin, morphine, myricetin, naringenin, quercetin, rhamnetin, rutin, tricine, and vitexin were found in control and polluted samples of studied taxa. Table 4 shows statistical analysis results of morphobiometrical, palynological and flavonoids studied data using SPSS by Tukey and Duncan tests.

Table 1. Seed morphobiometrical data of 10 collected Brassicaceae taxa from the Shazand (Arak) Thermal Power Plant area, Iran comparing to control

Taxa	SLM (mm) M±SD		SWM (mm) M±SD		SDM (mm)		SLM/SWM		NS%		AS%	
	C	P	C	P	C	P	C	P	C	P	C	P
Alyssum linifolium var. *linifolium*	1.6±0.06	1.5±0.08	0.97±0.07	0.97±0.03	0.22±0.01	0.2±0.03	1.65	1.54	92	84	8	16
Alyssum longistylum	2.14±0.01	2.05±0.07	1.51±0.07	1.61±0.06	0.66±0.04	0.56±0.09	1.42	1.27	97	94	3	6
Alyssum marginatum	2.26±0.08	1.8±0.05	1.56±0.04	1.3±0.06	0.54±0.04	0.5±0.02	1.45	1.38	99	98	1	2
Choriospora persica	2.61±0.11	2.56±0.11	2.22±0.22	2.25±0.13	0.75±0.05	0.74±0.09	1.17	1.14	94	81	6	19
Clypeola lappacea	2.05±0.14	1.67±0.11	1.77±0.06	1.47±0.06	0.58±0.09	0.46±0.07	1.15	1.13	94	67	6	33
Conringia perfoliata	2.23±0.14	1.88±0.09	1.13±0.05	0.80±0.04	0.94±0.06	0.46±0.02	1.97	2.35	97	90	3	10
Descurainia sophia	1.03±0.05	1.02±0.04	0.52±0.03	0.54±0.05	0.40±0.08	0.37±0.05	1.98	1.88	95	89	5	11
Goldbachia laevigata	2.60 ±0.13	2.57 ±0.06	1.58±0.07	1.56±0.11	1.30±0.10	1.30±0.15	1.64	1.65	80	75	20	25
Isatis kotschyana	5.50±0.18	4.50±0.18	1.94±0.13	1.63±0.06	1.79±0.13	1.40±0.15	2.83	2.76	87	74	13	26
Neslia apiculata	1.76±0.07	1.60±0.09	1.27±0.05	1.12±0.12	1.06±0.07	0.87±0.12	1.38	1.43	93	99	7	1

Note: C=control, P=polluted, SLM= seed length max., SWM= seed width max., SLM/SWM=seed length max/seed width max., SDM = seed diameter max., NS%= normal seed%, AS%= abnormal seed%.

Table 2. Palynological data of polluted samples of 10 collected Brassicaceae from the Shazand Thermal Power Plant area, Iran comparing to control using Muntezing's acetocarmine method

Taxa	Normal pollen grain						Abnormal pollen grain						Total fertility %		Total sterility %	
	Normality %		Fertility %		Sterility %		Abnormality %		Fertility %		Sterility %					
	C	P	C	P	C	P	C	P	C	P	C	P	C	P	C	P
Alyssum linifolium var. *linifolium*	80.2	65.3	100	90.3	0	9.7	19.8	34.7	95	39.4	5	60.6	99	72.6	1	27.4
Alyssum longistylum	71.8	53.6	98.2	96.6	1.8	3.4	28.2	46.4	81.8	69.2	18.2	30.8	93.6	66.07	6.4	33.93
Alyssum marginatum	89	50	100	91.6	0	8.4	11	50	80	75	20	25	97.8	83.33	2.2	16.67
Choriospora persica	79.1	69.9	100	100	0	0	20.9	30.1	80.5	30.6	19.5	69.4	95.9	79.1	4.1	20.9
Clypeola lappacea	90.8	89.8	100	100	0	0	9.2	10.2	25	92.9	75	71	93.1	90.6	6.9	9.4
Conringia perfoliata	97.9	87.4	100	100	0	0	2.1	12.6	0	34.8	100	65.2	97.9	91.8	2.1	8.2
Descurainia sophia	98.2	75.4	100	100	0	0	1.8	24.6	60	32.8	40	67.2	99.3	83.5	0.7	16.5
Goldbachia laevigata	96.4	95.1	100	99.1	0	0.9	3.6	4.9	75	16.7	25	83.3	99.09	95.08	0.91	4.92
Isatis kotschyana	95.3	49.2	100	93.8	0	6.2	4.7	50.8	0	16.4	100	83.6	95.3	54.5	4.7	45.5
Neslia apiculata	85.3	83.8	98.9	98.5	1.1	1.5	14.7	16.2	43.7	46.2	56.3	53.8	90.82	90	9.18	10

Note: C=control, P=polluted

Table 3. Pollen grain flavonoids data of ten studied Brassicaceae taxa from the Shazand Thermal Power Plant area, Iran comparing to control using 2-DPC and TLC methods

Taxa	*Alyssum linifolium* var. *linifolium*		*Alyssum longistylum*		*Alyssum marginatum*		*Choriospora persica*		*Clypeola lappacea*		*Conringia perfoliata*		*Descurainia sophia*		*Goldbachia laevigata*		*Isatis kotschyana*		*Neslia apiculata*	
Characters	C	P	C	P	C	P	C	P	C	P	C	P	C	P	C	P	C	P	C	P
Total flavonoids number	6	10	7	9	5	4	8	11	10	11	9	12	6	8	11	7	9	11	7	11
Flavonoid sulphates number	4	4	4	6	3	2	3	6	6	6	6	7	4	6	7	5	6	8	5	7
Flavon *C*-and *C*- /*O*-glucosides	2	6	3	3	2	2	5	2	3	3	3	4	2	1	4	2	2	3	2	4
Aglycones	1	0	0	0	1	1	0	3	1	2	0	1	0	1	0	0	1	0	0	0
Apigenin	-	-	-	-	-	-	++	+++	++	+++	+	++	+++	++	-	-	++	-	-	-
Chrysin	++	+	-	-	++	++	+++	++	++	++	+	++	++	++	++	++	++	+	++	++
Genistein	-	-	-	-	-	-	-	-	-	-	-	-	+++	+++	++	++	-	-	++	+++
Isorhamnetin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	±	±
Kaempferol	+++	++	+++	+	++	++	++	+++	++	+++	++	++	++	++	++	++	+++	++	++	+++
Luteolin	-	-	-	-	++	++	+	+	++	++	+	+	++	++	-	-	-	-	++	++
Morin	-	-	-	-	-	-	-	-	-	++	-	-	-	-	-	-	-	-	-	-
Myricetin	+	+	-	-	-	-	-	-	-	++	-	+	+++	++	++	++	++	-	-	-
Naringenin	-	-	-	-	-	-	-	-	++	+++	-	-	++	++	++	++	++	-	±	+
Quercetin	+++	+++	+++	+	+++	++	++	+++	++	+++	++	++	+++	++	++	++	++	++	++	++
Rhamnetin	-	-	±	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	±	±
Rutin	-	-	-	-	-	-	++	++	-	-	-	-	-	-	++	++	-	-	-	-
Tricin	-	-	±	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Vitexin	-	-	-	-	++	++	-	-	±	±	-	-	+	+	-	-	++	++	-	-

Note: Scored characters: - (non flavonoid=1), ± (UV absorbance < 0.1, rare concentration of flavonoid=2), + (few concentration of flavonoid=3), ++ (middle concentration of flavonoid=4), +++ (high concentration of flavonoid=5)

Also Table 5 shows selected significant parameters by ANOVA ($P \leq 0.05$).

Discussion

As Table 1 shows pollution have affected on seed dimensions in all of studied polluted samples. Seed dimensions have reduced in polluted plants comparing to control. But seed width has been exceptionally increased in *Alyssum longstylum* and *Descurainia sophia*. All of polluted sample fruits had more abnormal seeds number (without kernel, holed or wrinkled) in comparison with control (Figure 3). *G. laevigata* and *N. apiculata* polluted fruits had less abnormal seeds than control. Statistical analysis results in Tukey and Duncan tests showed that power plant pollutant had been caused normal seed number reduction in all of species exceptional *G. laevigata* and *N. apiculata* that confirmed Agrawal and Deepak (2003). SO_2 effects on soybean caused seed number reduction and consequently crop decreasing. Sprugel et al. (1980) showed using 0.09-0.79 µg/Lit SO_2 on soybean significantly caused seed weight and number reduction that mainly reduced crop (Sprugel et al. 1980; Reich and Amundson 1984).

Using Muntezing's acetocarmine pollen grain staining method revealed decreasing of fertility in all of ten studied polluted Brassicaceae taxa (Table 2). The method is one of the most widely used staining techniques for pollen viability estimation and shows sharp differences in smearing advanced pollen grains from abnormal ones. As a result, sharply stained pollen grains were considered as potential of fertile and viable ones, and partially stained, no stained, or weakly stained pollen grains were considered nonviable and sterile (Malayeri et al. 2011). As Table 2 shows the highest abnormality and sterility percentages pollen grain was observed in *I. kotschyana* in comparison with the same species control samples and also other polluted species. The results show stimulation and inhibition of these pollen characteristics depend on the plant species as well as on the pollutant and its concentration. Finally, this study indicates that air pollution can induce several abnormality and sterility in plant pollen grains.

Table 4. Statistical analysis results of seed and pollen morphobiometrical and pollen flavonoids data using SPSS by Tukey and Duncan tests ($P \leq 0.05$)

Test of homogeneity of variances	Levene statistic	DF	DF2	SIG.
Apigenin	.375	1	18	.548
Chrysin	.000	1	18	1.000
Genistin	.000	1	18	1.000
Isorhamnetin	.000	1	18	1.000
Luteolin	.000	1	18	1.000
Morin	5.062	1	18	***.037**
Myricetin	.375	1	18	.548
Naringenin	.375	1	18	.548
Rhamnetin	.050	1	18	.826
Rutin	.000	1	18	1.000
Tricin	1.013	1	18	.328
Vitexin	.000	1	18	1.000
Flavonoid Sulphates Number	.002	1	18	.961
Aglycones Number	2.796	1	18	.112
Flavonoid C-and C-/O-glucosides Number	.321	1	18	.578
Total Flavonoid Number	.361	1	18	.555
Apigenin concentration	.698	1	18	.414
Chrysin concentration	.004	1	18	.950
Genistin concentration	.184	1	18	.673
Isorhamnetin concentration	.000	1	18	1.000
Kaempferol concentration	5.684	1	18	***.028**
Luteolin concentration	.000	1	18	1.000
Morin concentration	5.062	1	18	***.037**
Myricetin concentration	.434	1	18	.518
Naringenin concentration	.129	1	18	.724
Quercetin concentration	.000	1	18	1.000
Rhamnetin concentration	1.531	1	18	.232
Rutin concentration	.000	1	18	1.000
Tricin concentration	1.013	1	18	.328
Vitexin concentration	.000	1	18	1.000
Seed length max.	.026	1	18	.874
Seed width max.	.000	1	18	.996
Seed length max/Seed width max.	.112	1	18	.742
Seed diameter max.	.172	1	18	.684
Abnormal seed percentage	5.878	1	18	***.026**
Abnormal pollen percentage	5.653	1	18	***.029**
Sterile pollen percentage	10.464	1	18	***.005**

Note: *Bold numbers significant ($P \leq 0.05$)

Table 5. Selected seed and pollen morphobiometrical and pollen flavonoids significant parameters by ANOVA ($P \leq 0.05$)

ANOVA		Sum of Squares	df	Mean square	F	Sig
Kaempferol concentration	Between Groups	.800	1	.800	4.800	***.042**.
	Within Groups	3.000	18	.167		
	Total	3.800	19			
Abnormal seed %	Between Groups	296.450	1	296.450	4.017	.060
	Within Groups	1328.500	18	73.806		
	Total	1624.950	19			
Abnrormal pollen grain%	Between Groups	1353.013	1	1353.013	7.220	***.015**
	Within Groups	3373.005	18	187.389		
	Total	4726.018	19			
Sterile pollen%	Between Groups	1204.818	1	1204.818	13.766	***.002**
	Within Groups	1575.343	18	87.519		
	Total	2780.161	19			

Note: *Bold numbers significant ($P \leq 0.05$)

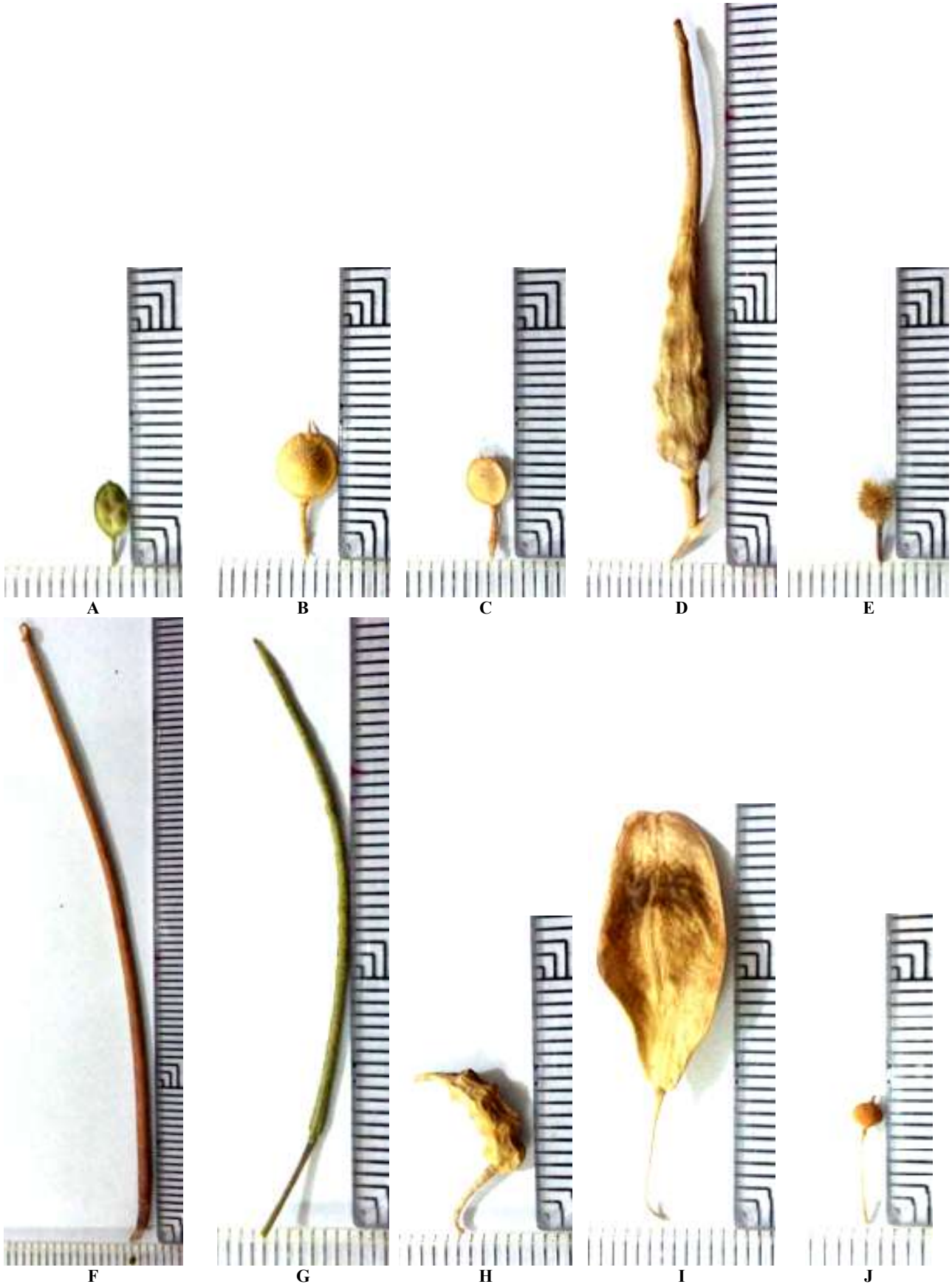

Figure 3. Showing fruits of 10 studied Brassicaceae taxa. A. *Alyssum linifolium* var. *linifolium*, B. *Alyssum longstylum*, C. *Alyssum marginatum*, D. *Choriospora persica*, E. *Clypeola lappacea*, F. *Conringia perfoliata*, G. *Descurainia sophia*, H. *Goldbachia laevigata*, I. *Isatis kotschyana*, J. *Neslia apiculata*. Strip = 1 mm

Phytochemical results in Table 3 show many changes in kind and number of flavonoids in polluted plants compared to control. The total flavonoids increasing were observed in polluted samples of *N. apiculata* and *A. linifolium*. Flavonoids number increase in polluted *Robinia pseudoacacia* grown under fluoride pollutant (Noori et al. 2009). Also using HPLC method on polluted pollen grain ethanolic extracts of *Lagerstroemia speciosa*, *Spartium junceum*, *Petunia hybrid* and *Thuja orientalis* showed that air pollution caused high-level flavonoids accumulation in polluted samples in comparison with control (Rezanezhad 2012). Flavonoid sulfates number in *A. longistylum*, *C. persica*, *C. perfoliata*, *D. sophia, I. kotschyana* and *N. apiculata* polluted pollen grains were more than control while these flavonoids in *A. marginatum* and *G. laevigata* were less than and *A. linifolium* and *C. lappacea* did not show any changes. As our TLC results show, some flavonoids appeared in polluted samples while disappearance others were observed. Quercetin and kaempferol were found in all of control and polluted samples while rhamnetin lacked. Tricin was just found in polluted *A. longistylum* sample. Chrysin found in all of control and polluted samples except *A. longistylum*. Presence of morin and myricetin in polluted *C. perfoliata* and *I. kotschyana* and absence in their control show creation or deletion of flavonoids under pollutant effects. Studies on eight angiosperm plants collected from Shazand Oil Refinery area, Iran using two-dimensional paper and thin layer chromatography showed flavonoids kind and number changing in polluted plants comparing to control. Also, kaempferol appearance in polluted *Amaranthus chlorostachys* samples comparing to control was observed (Kamalabadi 2013).

Robles et al. (2003) studied phenols and flavonoids in Aleppo pine needles as bioindicators of air pollution. Their results showed that total flavonoids are useful bioindicators for ozone pollution (significant positive correlations between total flavonols and ozone pollution). Sulfur dioxide pollution is distinguished by low concentrations in quercetin, isorhamnetin, and kaempferol (significant negative correlations between these simple flavonols and the concentrations of SO2).) The work confirms strong interest of using the phenolic compounds of *Pinus halepensis* as biological indicators of air quality (Robles et al. 2003). Rubin et al. (1983) found induction of isoflavonoid production in *Phaseolus vulgaris* leaves by ozone, sulfur dioxide and herbicide stress (Rubin et al. 1983). Total flavonoids reduction was observed in treated plants with heat and SO_2 (Lee et al. 2002; Lavola, 1997). Increasing total flavonoids and phenolic compounds were observed in collected *Catharanthu*s roseus and *Ocimum sanctum* from NO_2 and SO_2 polluted area. These plants can be used as bioindicators (Qayoom et al, 2009). Increasing or reduction of flavonoids under air pollutant can show pollution effects on flavonoids. Phytochemical changes in aluminum smelter industry area vegetation were observed with appearance or disappearance of flavonoids in fluoride polluted plants (Noori et al. 2009). Statistical analysis results of morphobiometrical and flavonoids data using SPSS by Tukey and Duncan tests in Table 4 show morin existence and morin and kaempferol concentrations, abnormal seed and pollen percentages and sterile pollen% are significant ($P \leq 0.05$). Also, Table 5 shows selected seed morphobiometrical, palynological and pollen phytochemicals significant parameters by ANOVA method ($P \leq 0.05$) and confirms that kaempferol concentration, abnormal seed and pollen grains percentages and sterile pollen% are three significant parameters. These results have coincided on Sprugel et al. (1980) about seeds, Noori et al. (2014) about pollen and Kamalabadi (2013) about flavonoids. It is known that plant flavonoids pattern depends on genetics factors and ecological conditions.

Results of the study confirmed that pollen grains provide essential information on biological impact of pollutants and they are good candidates for biomonitoring the atmospheric and edaphic pollutions. As Ruffin et al. (1983) studied effects of some environmental gaseous pollutants on pollen-wall proteins of certain airborne pollen grains. They used pure pollen grains of *Ulmus pumila*, *Quercus rubra*, *Pinus taeda*, and *Festuca elatior* were used as a biological screen to test the effects of certain commonly occurring atmospheric pollutants on pollen wall protein (Ruffin et al. 1983). Also, Rezanejad (2009) studied air pollution effects on structure, proteins, and flavonoids in pollen grains of *Thuja orientalis*.

Pollutions such as power plant pollutant are caused by different environmental effects. The most pollutants of power plant are SO_2, NO, NO_2, CO, and CO_2 that affected on ecosystem and plant vegetation. These atmospheric pollutants affect pollen morphobiometry, fertility, and phytochemistry. Their effective on plants caused different morphological, physiological and phytochemical responses to pollutants that reduced pollen and seed production and their viability and fertility. Changes in flavonoids kind and number especially flavonoids sulfates production or increasing in polluted plant in comparison with control plant defensive reactions to pollutants. It seems that environmental pollution reduced plant resistance against catching disease and pest attack and increased plant sterility. Then studying pollen characters such as viability, morphobiometery, their phenols and flavonoids compounds and also seed production and characters can be used as air quality bioindicators.

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