

Leaf surface structure alterations due to particulate pollution in some common plants

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Abstract The plant foliar surface is the most important receptor of atmospheric pollutants. It undergoes several structural and functional changes when particulate-laden air strikes it. In the present investigation, ten annual plant species viz., *Abelmoschus esculentus*, *Celosia cristata*, *Coleus blumei*, *Cyamopsis tetragonolobus*, *Gomphrena globosa*, *Impatiens balsamina*, *Ocimum sanctum*, *Phaseolus vulgaris*, *Solanum melongena*, and *Zinnia elegans* were studied for their growth parameters and leaf morphological features. They were subjected to dust experimentally for 60 days. The micro-morphological traits like wax, cuticle, epidermis, stomata, and trichomes were observed under light and scanning electron microscopes. Remarkable differences in the growth parameters and micro-morphological features were recorded in the dust-treated plants when compared to the respective controls. The reduction in growth parameters, the size of epidermal cells, and stomata were reduced and cuticle damage was also observed. The relative proportion of fine particles, which play a major role in hampering the overall growth of a plant, was higher in comparison to ultra-fine and coarse particles.

Keywords Particulate · Morphological features · Plant foliage · Ultra fine · Coarse particles · Ultra-structure · Urban dust

1 Introduction

The particulate pollution has always been a matter of great concern because of its adverse effect on humans and plant populations. In the present global environmental scenario, the problem has become increasingly severe. The automobile exhaust emitted by the moving vehicles on the roads are the main producers of these particles. The behavior of suspended particulates in the atmosphere may be determined by the particles with a short life span, whose size ranges from fine (soot and diesel particles) to coarse (road dust, soil) type (QUARG 1996).

It is already known that trees improve urban air quality (Freer-Smith et al. 1997). Because of the roughness and large contact area, the foliage of plants filter numerous solid particles and can be effective in measuring the damaging effects of particulate pollution (Meusel et al. 1999). The symptoms of injury due to air-borne particulates first appear in the leaf tissue at a macroscopic level and the affected cells often indicate the causative agent. It is therefore useful to consider the macro and micro-morphology of a leaf to assess the impact of dust pollution (Lohr and Pearson-Mims 1996). The roughness on the leaf may appear due to some morphological parameters like cuticular ornamentations, raised epidermal cell boundaries, stomatal ledges, trichomes and, overall, the epicuticular and cuticular waxes (Pal et al. 2002).

Vegetation is an effective indicator of the overall impact of air pollution, and the effect observed is a time-averaged result that is more reliable than the one obtained from direct determination of the pollutant in air over a short period. Although a large number of trees and shrubs have been identified and used as dust filters to check the rising urban dust pollution level (Lorenzini et al. 2006), leaf traits can be used as a tool for estimating the state of the

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environment, and further research is required to consider the response of leaf surface characters to the particle size (Kosiba 2008).

The objectives of this study are to examine changes in the leaf morphological traits and the overall growth performance of the plants due to the toxicity of urban dust particles.

2 Materials and methods

Ten annual plant species selected for this study were *Abelmoschus esculentus* (Linn.) Moench., *Celosia cristata* Voss., *Coleus blumei* Benth., *Cyamopsis tetragonolobus* (Linn.) Taub., *Gomphrena globosa* Linn., *Impatiens balsamina* Linn., *Ocimum sanctum* Linn., *Phaseolus vulgaris* Linn., *Solanum melongena* Linn. and *Zinnia elegans* Jacq. The relative proportion of ultra-fine, fine, and coarse particles was determined in the sample of urban dust applied on the leaf surfaces of the studied plants. The seedlings of each plant species in ten replicates were planted in 1-m² plots (six seedlings per plot) in the Eco-education garden of National Botanical Research Institute, Lucknow, India. At 4–6-leaf stage, each plant was sprayed with 5 g urban dust daily. Dust samples for the spray were collected from the roadside and the spraying was done with a hand rotatory duster (Code No. ARD manufactured by Orient Ltd., India). The unsprayed plants of a given species served as the control. Each plant species was randomly harvested from both the control and dust-treated plots at 30, 45, and 60-day intervals.

The growth parameters such as root, shoot length (cm), number of leaves, and leaf area (cm²) per plant were determined. Flowering and fruiting behavior and the micro-morphological characters of leaves were studied with light microscope and the surface structural properties were studied with scanning electron microscope.

3 Observations

3.1 Light microscopy

Mature and healthy leaves from the control and dust-treated plants were selected for light microscopic studies. Cuticles were separated from leaves by scraping with a safety-razor blade the upper and lower surfaces separately, (Kulshreshtha et al. 1980). The cuticles were washed with water, stained with aqueous safranin, mounted in pure glycerin, and semi-permanent slides were made. The plant characters observed were size and frequency of epidermal cells, length and width of stomata and trichomes (per μm).

3.2 Scanning electron microscopy

Small strips (about 1 cm²) were trimmed from areas between the margin and midrib of leaves, fixed in FAA (formaldehyde: acetic acid: alcohol, 9:0.5:0.5), dehydrated through ethanol series (from 30% to absolute), and dried in a critical point drier using liquid CO₂ at 1,075 psi and 31.4°C pressure and temperature, respectively. Two pieces of leaf (about 0.5 cm²) were cut from the dried strips and mounted on stubs, with double-sided adhesive tape taking care to expose adaxial and abaxial surfaces side by side on the same stub. The specimens were coated with a thin conductive film of gold (about 200Å), in an ion sputter coater (JFC 1100). Coated specimens were examined and photographed under a scanning electron microscope (Philips XL-20, The Netherlands) at an accelerating voltage of 10 kV, at the magnification range of 200–1,000 \times .

3.3 Measurement of dust particles

Particle size and their distribution in urban dust were measured by suspending dust samples in water (w/v). A drop of this suspension after thorough shaking was mounted on a slide beneath a cover slip. Measurement was done by calculating the maximum diameter of 100 randomly chosen particles in each of five drops per suspension, with the help of micrometers, under a light microscope (Leica ATC 2000).

4 Results and discussion

A comparison of growth parameters of the dust-treated and the control plants showed a reduction in plant growth, no of leaves, and leaf area in the former. The maximum effect was observed in *Gomphrena globosa*, where the flowering time was considerably delayed in the treated plants. The dust-treated plants of *Abelmoschus esculentus*, *Cyamopsis tetragonolobus*, *Phaseolus vulgaris*, and *Solanum melongena*, produced lesser number of fruits as compared to the untreated ones. The number of root nodules in *Cyamopsis tetragonolobus* and *Phaseolus vulgaris* were decreased with the duration for which the plants were sprayed with dust. Unlike the inhibition of shoot length, area of leaflets and internodal elongation due to pollution, as observed by Indhirabai et al. (1989) was not confirmed in this study. The cement kiln dust has been reported to decrease height, phytomass, and net productivity (Prasad et al. 1991).

While studying the micro-morphological parameters, it was observed that the frequency of epidermal cells and stomata had increased considerably in the dust-sprayed plants (Table 1). The significant reduction in epidermal cell size and stomata resulted due to inhibited cell

Table 1 Micro-morphological characters of leaf surfaces of the studied plants

S. N.	Plant species	Dust-treated										Control									
		US	LS	US	LS	US	LS	US	LS	US	LS	US	LS	US	LS	US	LS	US	LS	US	LS
		Epid. cell size (μm)	Stom. freq. (\pm SD)	Size (μm)	SI	Ng. (Length.)	Gt. (Diameter)	Epid. cell size	Stom. freq. (\pm SD)	Stom. size	SI	Ng. (Length.)	Gt. (diameter)	Epid. cell size	Stom. freq. (\pm SD)	Stom. size	SI	Ng. (Length.)	Gt. (diameter)		
1.	<i>Abelmoschus esculentus</i> (Linn.) Moench	US	64 × 48	24 ± 0.14	40 × 28	15	154	80 × 68	28 ± 0.06	52 × 32	14	143	–	80 × 68	28 ± 0.06	52 × 32	14	143	–		
		LS	80 × 56	42 ± 0.08	36 × 28	23	167	96 × 60	32 ± 0.01	48 × 32	21	161	–	96 × 60	32 ± 0.01	48 × 32	21	161	–		
2.	<i>Celosia cristata</i> Voss.	US	116 × 40	62 ± 0.02	40 × 22	39	–	128 × 72	26 ± 0.14	56 × 32	32	–	–	128 × 72	26 ± 0.14	56 × 32	32	–	–		
		LS	120 × 44	70 ± 0.04	48 × 32	43	–	144 × 76	28 ± 0.10	48 × 36	27	–	–	144 × 76	28 ± 0.10	48 × 36	27	–	–		
3.	<i>Coleus blumei</i> Benth.	US	132 × 96	–	–	–	272	156 × 108	–	–	–	280	–	156 × 108	–	–	–	280	–		
		LS	128 × 64	48 ± 0.06	36 × 24	25	248	104 × 56	40 ± 0.24	44 × 32	21	260	–	104 × 56	40 ± 0.24	44 × 32	21	260	–		
4.	<i>Cyamopsis tetragonolobus</i> (Linn.) Taub.	US	92 × 48	46 ± 0.04	32 × 28	32	–	100 × 60	22 ± 0.04	36 × 28	38	–	–	100 × 60	22 ± 0.04	36 × 28	38	–	–		
		LS	100 × 56	62 ± 0.12	36 × 28	27	–	128 × 72	54 ± 0.16	40 × 28	42	–	–	128 × 72	54 ± 0.16	40 × 28	42	–	–		
5.	<i>Gomphrena globosa</i> Linn.	US	160 × 120	14 ± 0.01	56 × 36	23	1,080	188 × 44	8 ± 0.02	64 × 40	18	800	–	188 × 44	8 ± 0.02	64 × 40	18	800	–		
		LS	136 × 156	13 ± 0.07	52 × 36	30	840	180 × 104	10 ± 0.00	56 × 40	19	840	–	180 × 104	10 ± 0.00	56 × 40	19	840	–		
6.	<i>Impatiens balsamina</i> Linn.	US	96 × 64	22 ± 0.12	44 × 28	15	–	112 × 72	14 ± 0.16	48 × 32	12	–	–	112 × 72	14 ± 0.16	48 × 32	12	–	–		
		LS	80 × 64	40 ± 0.18	40 × 24	13	–	152 × 80	20 ± 0.08	44 × 28	17	–	–	152 × 80	20 ± 0.08	44 × 28	17	–	–		
7.	<i>Ocimum sanctum</i> Linn.	US	92 × 48	22 ± 0.08	24 × 16	13	292	108 × 56	15 ± 0.22	36 × 24	18	272	44	108 × 56	15 ± 0.22	36 × 24	18	272	44		
		LS	108 × 36	40 ± 0.00	32 × 24	33	298	108 × 68	18 ± 0.18	32 × 24	18	320	64	108 × 68	18 ± 0.18	32 × 24	18	320	64		
8.	<i>Phaseolus vulgaris</i> Linn.	US	132 × 92	30 ± 0.10	36 × 24	24	–	160 × 144	30 ± 0.06	48 × 28	27	–	48	160 × 144	30 ± 0.06	48 × 28	27	–	52		
		LS	132 × 40	48 ± 0.04	44 × 28	29	–	168 × 72	48 ± 0.02	48 × 36	24	–	44	168 × 72	48 ± 0.02	48 × 36	24	–	56		
9.	<i>Solanum melongena</i> Linn.	US	76 × 40	30 ± 0.04	32 × 28	26	Stellate	112 × 48	20 ± 0.10	32 × 28	23	Stellate	–	112 × 48	20 ± 0.10	32 × 28	23	Stellate	–		
		LS	88 × 48	38 ± 0.04	36 × 28	32	Stellate	120 × 48	35 ± 0.06	32 × 28	41	Stellate	–	120 × 48	35 ± 0.06	32 × 28	41	Stellate	–		
10.	<i>Zinnia elegans</i> Jacq.	US	72 × 36	29 ± 0.02	56 × 28	21	113	124 × 80	21 ± 0.06	56 × 32	26	136	–	124 × 80	21 ± 0.06	56 × 32	26	136	–		
		LS	84 × 76	26 ± 0.12	44 × 32	20	136	104 × 44	19 ± 0.10	60 × 32	22	120	–	104 × 44	19 ± 0.10	60 × 32	22	120	–		

Epid Epidermis, Stom freq Stomatal frequency, SI Stomatal index, Ng. non-glandular trichome, Gr Glandular trichome, US Upper surface, LS Lower surface, SD Standard deviation

elongation. Kulshreshtha et al. (1980) had previously reported significant decrease in the size of epidermal cells, stomata, and trichomes per unit area in hydrogen fluoride and carbon particulate-polluted populations of *Jasminum sambac* collected from a bangle factory complex. In this study, the deposition of dust on upper leaf surface was not uniform and more than 75% of stomata were found clogged in *A. esculentus*, *C. blumei*, *S. melongena*, and *Z. elegans*. It is important to mention that the dust loading on leaves may reduce plant growth (Bender et al. 2002) through its effect on leaf gas exchange (Ernst 1982), occlude stomata (Hirano et al. 1995), reduce photosynthetically active radiations and increase the leaf temperature (Naidoo and Chirkoot 2004).

In the case of *A. esculentus*, *C. blumei*, *C. tetragonolobus*, *G. globosa*, *S. melongena*, and *Z. elegans*, it was observed that the presence of trichomes plays a significant role in trapping the dust particles and our observations support the views of Monn et al. (1995). The particles enter the leaf through stomatal openings and their toxicity may disturb the physiological activity of plants (Farmer 1993). This might be the reason why the morphological parameters show significant differences in their size and frequency (Table 1). The presence of particles in stomata can also be a cause of increase in the total concentration of metal elements in the foliage (Grantz et al. 2003; Mankovska et al. 2004). The inhibition in plant growth, rate of photosynthesis, late flowering, and the hormonal imbalance may be due to the deficiency of essential nutrients in the polluted plants (Farooqui et al. 1995).

In SEM study, epicuticular wax was observed in the form of crust (*S. melongena*, Fig. 1a) or it could be star-

shaped (*C. tetragonolobus*, Fig. 2a) in control plants, whereas in the dust-treated plants (Figs. 1b, 2b) it was insignificant, giving the appearance of aggregate patches. It had also been reported earlier that under stress conditions plants produce more wax than control (Hollenbach et al. 1997). The structure and morphology of epicuticular waxes is a reliable indicator of plant health (Neinhuis and Barthlott 1998) and to a great extent, regulate the resistance to pollution stress. Sauter and Pambor (1989) observed increased degradation of epicuticular wax in spruce and fir (*Abies alba*) due to deposition of road dust. Changes in leaf wettability, rate of transpiration, and loss of solutes from leaf cells are some of the effects that result from disruption of the epicuticular/epistomatal wax layer (Bystrom et al. 1968).

Smooth cuticle, sometimes striated, sinuous epidermal cell walls and clear cell boundaries were observed in control plants of *A. esculentus*, (Fig. 3a) *C. tetragonolobus* (Fig. 2a, c), *G. globosa*, *P. vulgaris*, and *Z. elegans*. Stomata were in level with the epidermal cells in *I. balsamina* and *S. melongena* (Fig. 1c) and characteristic large-sized stellate trichomes were recorded in *S. melongena* (Fig. 1a). In the dust-treated plants characteristic wrinkles appeared and sinuous nature of epidermal cells and distinct cell boundaries were completely lost on the cuticle (Fig. 2d). Stomata, slightly raised from rest of the cells, were often filled with dust particles (Figs. 1d, 3b), and at some places were also clogged. Patches of injured cells were also visible in *C. tetragonolobus* and *G. globosa* on their stomatal ledges. The trichomes in *A. esculentus*, *C. blumei*, *G. globosa*, *O. sanctum*, *S. melongena* (Fig. 1b), and *Z. elegans* lost their turgidity and showed irregular patterns on their surface.

Fig. 1 Scanning electron microphotographs of leaves of *Solanum melongena* Linn. Showing **a** stellate trichomes in control, **b** stellate trichomes that have lost turgidity in treated leaves, **c** clear and open stomata in control, **d** dust trapped closed stomata

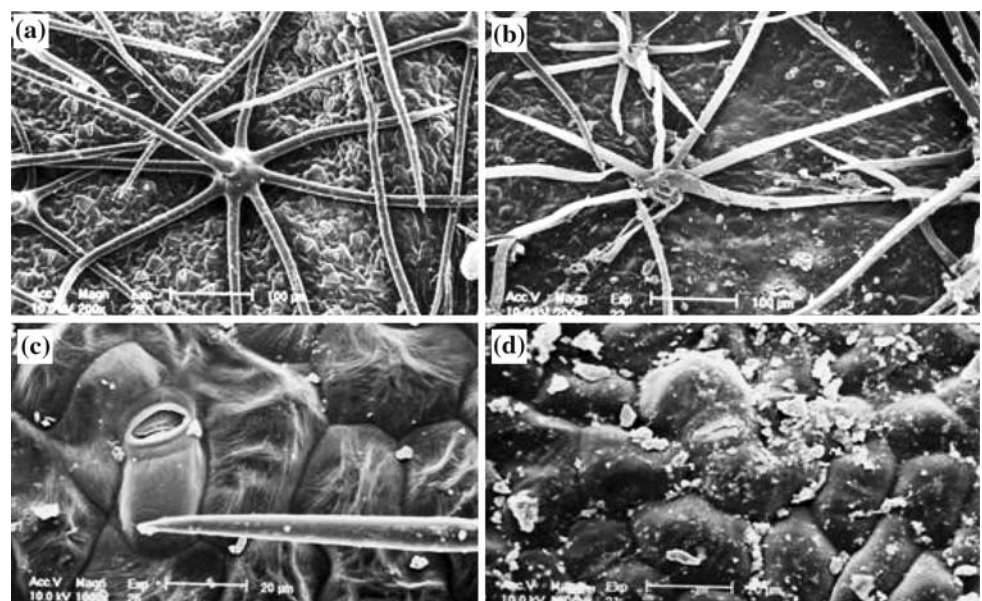


Fig. 2 Scanning electron microphotographs of *Cyamopsis tetragonolobus* (Linn.) Taub. showing **a** healthy epidermal cells and stomata on lower surface in control, **b** closed stomata in treated leaves, **c** epidermal cells on upper surface of leaf in control, **d** increased epidermal cell number in the dust-treated leaves

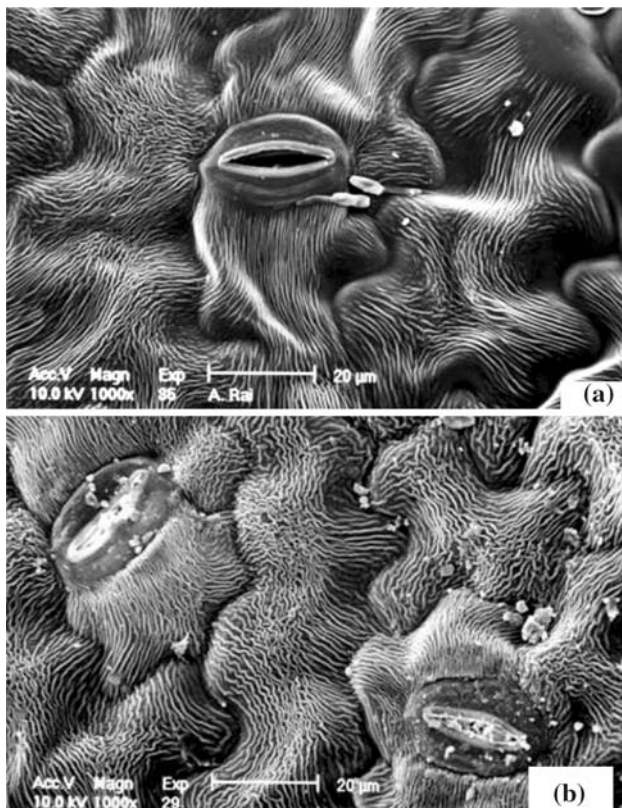
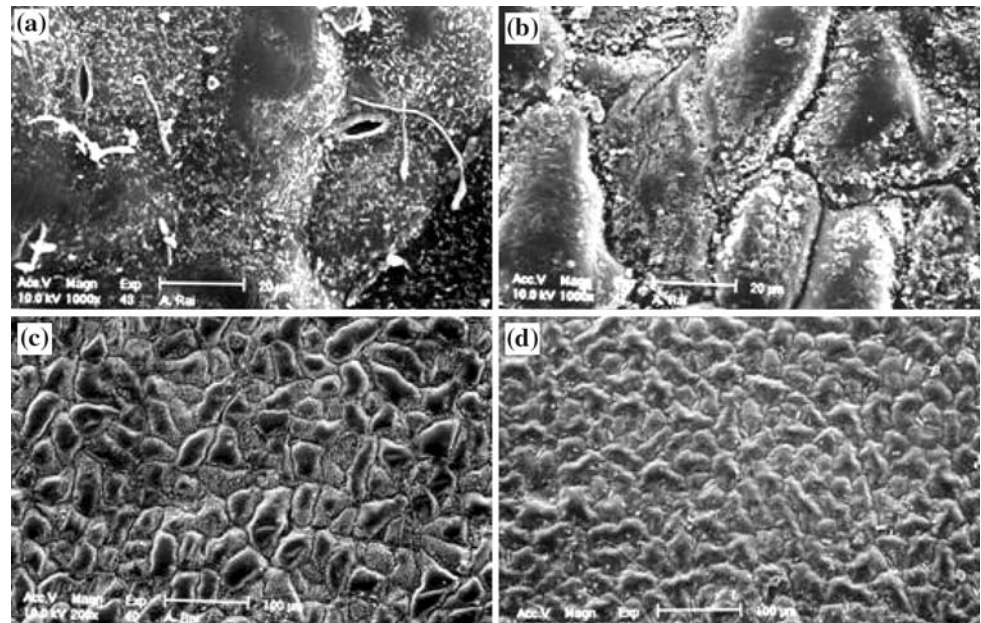


Fig. 3 Scanning electron microphotographs of *Abelmoschus esculentus* (Linn.) Moench. showing **a** clear stomata and linear striations in control, **b** dust-filled stomata and slightly disturbed striations in the dust-treated leaves

Urban dust consists of gray, irregular, spherical, and elongated particles. Almost 75% of the particulate mass contained particles having an aerodynamic diameter of

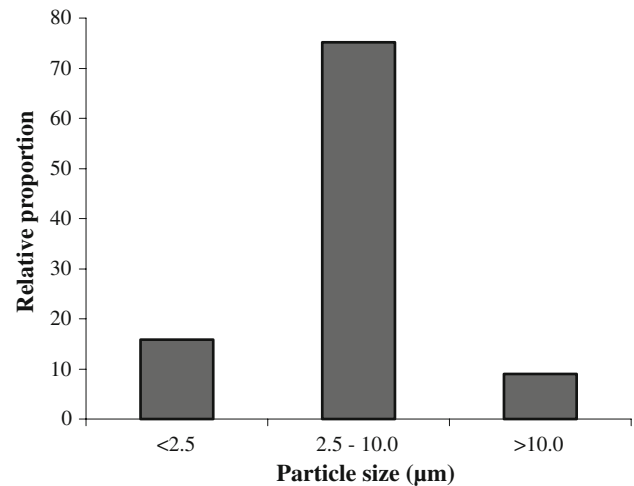


Fig. 4 Relative proportion of the particles of different sizes present in the urban dust

2.5–10 µm, in comparison to ultra-fine (less than 2.5 µm) and coarse particles (more than 10 µm) (Fig. 4). It was also noticed that the particles larger than the stomata opening generally pile up on the pore, while fine particles clog the stomata, affecting the gaseous exchange process and in turn affecting photosynthesis, water retention, respiration, and overall growth of the plants. As the roadside plants covered with dust also suffer from water deficiency, a well-developed epicuticular wax layer may be crucial in protecting them from water loss, and any change in the original morphological structure make these plants more sensitive to water loss (Saneoka and Ogata 1987).

It is clear from the observations that the local annuals subjected to urban dust spray showed reduced growth and

remarkably altered leaf surface structures. These changes in micro-morphological features of leaves show the stress conditions of the plant and can serve as an indicator of dust pollution. We argue that changes in leaf surface ultra structure may help the plant grow well in that stress condition.

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