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RESEARCH ARTICLE

EFFECTS OF AUTO EXHAUST POLLUTION ON FOLIAR MORPHOLOGICAL STRUCTURE OF ROAD SIDE SHRUB SPECIES, *BOUGAINVILLEA SPECTABILIS*

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ABSTRACT

Auto exhaust pollution serve as the major driver of the deteriorating air quality in Rewa city. This study aims to investigate the effects of auto exhaust pollution on foliar morphological structure of road side ornamental shrub species, *Bougainvillea spectabilis*. Increased number of stomata and epidermal cells per unit area in leaf samples of *Bougainvillea spectabilis* collected from polluted sites than those from control sites is observed by light microscopic technique. Analysis of data revealed that, as compared to the leaves from control, the length and width of guard cells and epidermal cells reduced significantly in leaves of polluted sites. These changes in foliar morphological structures could be as indicator of auto exhaust pollution or environmental stress.

INTRODUCTION

Air pollution is a major problem arising mainly from urbanization, growing population and industrialization. The Auto exhaust pollution or vehicular pollution is a major environmental concern, particularly in the developing countries and in their major cities. The problem of auto pollution is much more increased due to old and poorly or not maintained petrol and diesel vehicles running on the roads which releases variety of pollutants particularly benzene, carbon monoxide , organic compounds, oxides of nitrogen and sulphur and suspended particulate matters like ultra fine primary particles, smoke, metals and dust. Also the ultra fine particles when released quickly coagulate to form larger particles, through reaction with other pollutants like ammonia, sulphur dioxide etc (Street *et al.,* 1996). Many researches carried out to highlight the effect of air pollution on micro-morphological (Palaniswamy *et al.,* 1995; Morison, 1998; Aggarwal, 2000 and Rai *et al.,* 2015), anatomical (Salgare and Rawal, 1990; Acharekar and Salgare, 1991) and biochemical (Pratibha and Sharma, 2000; Karthiyayini *et al.,* 2005; Ramakrishnaiah and Somashekar, 2003; Gupta *et al.,* 2009) parameters of different plant species at various places. Plant species, particularly trees and shrubs, are important sinks for absorbing many gases, particulates, aerosols and airborne pollutants (Gajghate and Hassan 1999). The road side plants play a significant role in assimilation and accumulation of pollutants and act as efficient interceptors of airborne pollutants. Adverse impacts of urban air pollution on leaf structure of plants have been studied by various researchers (Kulshreshtha *et al.,* 1994a, 1994b; Hirano *et al.,* 1995; Carreras *et al.,* 1996; Dineva, 2006; Rai and Kulshreshtha, 2006; Sher and Hussain, 2006; Amulya *et al.,* 2015; Pawar, 2016). Auto exhausts are the main source of pollution in ambient atmosphere of the Rewa city. This study was undertaken to assess the foliar morphological changes caused by Auto exhaust pollution on number and size of stomata and epidermal cells in the leaves of a roadside shrub species, *Bougainvillea spectabilis*.

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MATERIALS AND METHODS

Selection of Site

The present study was conducted in Rewa city, which is situated on the north east border of Madhya Pradesh, central part of India. It is located at 24.53^o latitude north and 81.3^o longitude east and 316 meters above mean sea level (MSL), with a total geographical area 6,240.06 sq.km having a population about 2.36 lakhs.

Selection of plant

A common road side shrub species,*Bougainvilleaspectabilis* growing at five polluted sites viz; Stadium chowk, P.K. school, Samanchowk, Dhobiatanki, Transport nagar and Civil lines of Rewa city, M.P.(India) and A.P.S. university, Rewa campus as control site was selected for study of foliar epidermal features of leaves.

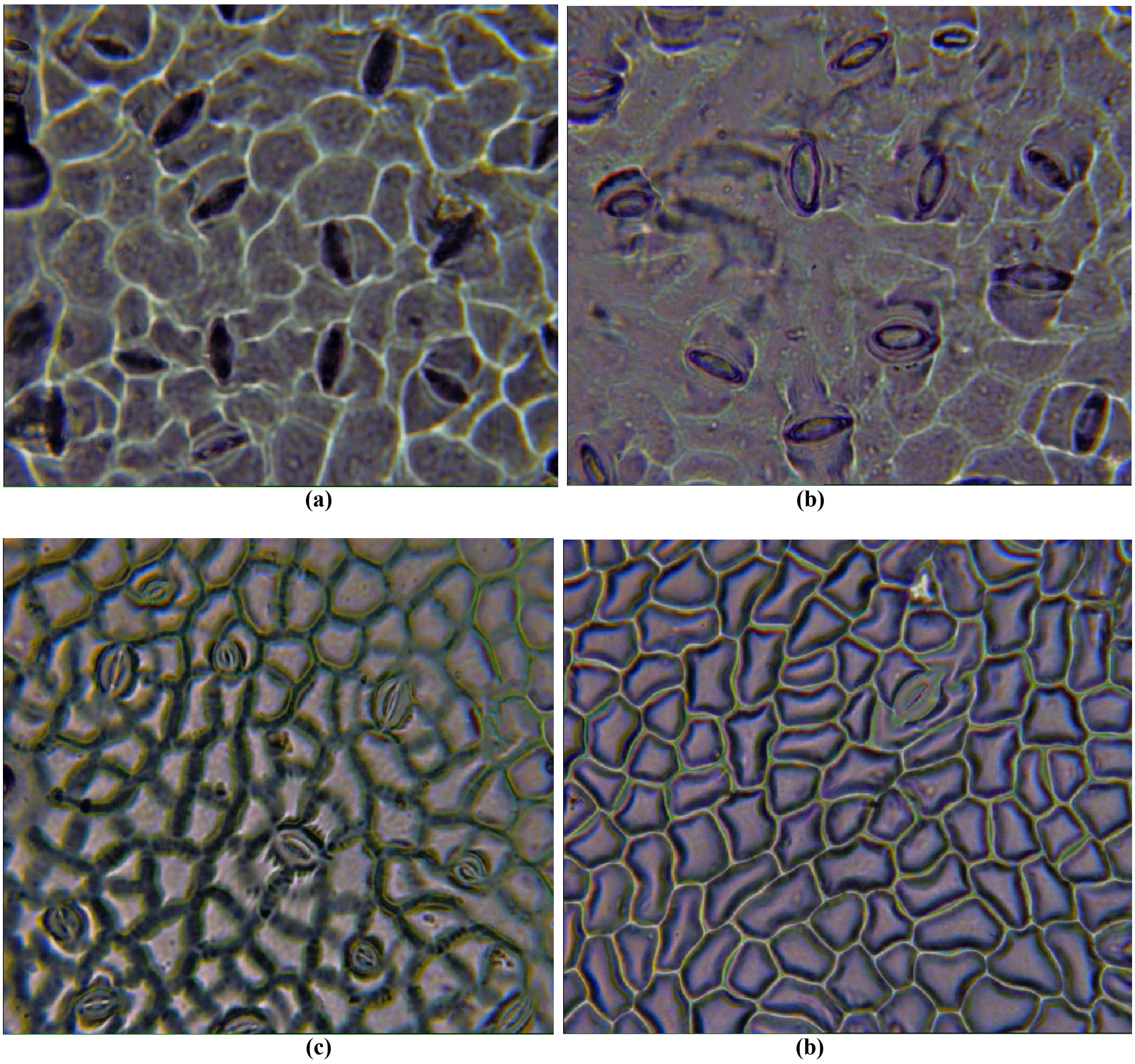

Fig. 1. Effect of air pollution on foliar-morphological structures on leaves of*Bougainvillea spectabilis* (left side: control site; right side: polluted site): (a) and (b) lower foliar surface (c) and (d) upper foliar surface

Microscopic studies

Foliar morphological characteristics of leaves were studied with light microscope. For light microscopic study (LMS), fresh new and old leaf samples of *Bougainvillea spectabilis* were collected from control and polluted sites during March to July 2016 and were properly washed with tap water and deiodenized water to remove all loose dust particles from their surface. Slides were prepared as per lasting impression method for counting of stomata and epidermal cells. In this method, one square centimeter on surface of leaf was painted by a thick patch of clear or transparent nail polish. Nail polish is allowed to dry completely then a piece of clear cellophane tape is taped to the dried nail polish patch by a carton sealing tape. Gently, peeled out or take out the nail polish patch by pulling a corner of the tape and the finger nail polish along with the leaf peel. This leaf impression was taped on slides and labeled as abaxial and adaxial surface. Leaf impression was examined under 400 x total magnifications by light microscope ("Motic Images plus 2.0 ML" software). Number of stomata and epidermal cells were counted per square millimeter area. Length and width of epidermal cells and stomata guard cells of a leaf were measured in µm with the help of ocular micrometer under high power magnifications by micrometry i.e. "Stage-ocular micrometry".

Stomatal index is calculated by the formula of Salisbury (1927):

$SI = S / (E+S) \times 100$
Stomatal frequency is calculated by formula:
$SF = (S/E) \times 100$

Where, S= Average number of stomata and E= Average number of epidermal cells.

RESULTS AND DISCUSSION

Table-1 shows average number of stomata, length and width of guard cells of old and new leaves of *Bougainvillea spectabilis* growing at polluted and controlled sites of Rewa city. Results clearly indicated an increased number of stomata on ventral surface of both new and old leaves at polluted site as compared to control site. However, this increase was statistically insignificant (Table 2). The dorsal surface of new leaves has shown significantly decreased number of stomata at polluted sites. Table-1 also shows length and width of guard cells of *Bougainvillea spectabilis* of both dorsal and ventral surface of old and new leaves growing in polluted and controlled sites.

Table 1. Average number of stomata (per mm^2), length (µm) and width of guard cells (µm), stomatal frequency (%) and stomatal index (%) of *Bougainvillea spectabilis* leaves growing at polluted and controlled sites of Rewa City

Leaf Surface	Stomata characteristics	Polluted		Controlled	
		New	Old	New	Old
Dorsal	LGC	10.2 ±5.08	-	31.2 ±6.51	36.9 ±8.30
	WGC	27.3 ±11.42	-	20.1 ±5.66	28.8 ±7.92
	NOS	1.5 ±1.05	-	8.3 ±2.20	7.0 ±2.0
	SF	1.67	-	11.54	12.58
	SI	1.64	-	10.34	11.18
Ventral	LGC	36.0 ±6.93	33.6 ± 6.89	24.3 ±5.73	24.3 ±7.95
	WGC	26.1 ±7.49	48.3 ±7.80	34.2 ±5.69	17.7 ±6.7
	NOS	12.9 ±2.43	11.2 ±1.94	12.3 ±2.21	9.8 ± 2.65
	SF	13.55	15.58	13.92	15.44
	SI	11.94	13.39	12.22	13.37

LGC= Length of guard cells; WGC= Width of guard cells; NOS = Number of stomata; SF= Stomatal frequency; SI = Stomatal index

Table 2. Values of 't' test between number of stomata and size of guard cells of *Bougainvillea spectabilis* leaves of polluted and control sites of Rewa city

Leaf surface	Stomata characteristics	Nature of leaves	
		New	Old
	LGC	t=8.042****, P<0.0001	_
Dorsal	WGC	t=1.786*, P=0.0909	_
	NOS	t=8.821****, P<0.0001	_
Ventral	LGC	t=4.115***, P=0.0007	t=2.795**, P=0.0120
	WGC	t=2.723**, P=0.0139	t=9.411****, P<0.0001
	NOS	t=0.5776, P=0.5707	t=1.348, P=0.1944

LGC = Length of guard cells; WGC= Width of guard cells; NOS = Number of stomata;
* Significant: 't' value at 18 d.f. on 0.05% level is 1.734

Table 3. Average number (per mm^2), length (µm) and width (µm) of epidermal cells of *Bougainvillea spectabilis*leaves growing at polluted and controlled sites of Rewa City

Leaf Surface	Epidermal cells	Polluted		Controlled	
		New	Old	New	Old
	LEC	22.2±6.81	31.8 ±5.33	15.6 ± 6.60	25.2 ± 8.62
Dorsal	WEC	39.0±6.17	40.8 ±6.82	33.3 ± 6.99	37.8 ± 8.27
	NEC	89.5±6.67	75.6 ±6.08	71.9±10.84	55.6±12.02
	LEC	25.8±6.96	26.7 ±6.07	20.4 ±7.45	15.3 ±5.37
Ventral	WEC	27.9±7.21	36.0 ±6.63	30.0±12.72	31.5 ±5.87
	NEC	95.2±8.92	71.9 ±8.03	88.4±7.33	63.5±9.05

LEC = Length of epidermal cells; WEC = Width of epidermal cells; NEC = Number of epidermal cells

Table 4. Values of't' test between number and size of epidermal cells of *Bougainvillea spectabilis* leaves of polluted and control sites at Rewa city

Leaf Surface	Epidermal cells	Nature of leaves	
		New	Old
	LEC	t= 2.201**, P=0.0410	t=2.059*, P=0.0542
Dorsal	WEC	t=1.933*, P=0.0691	t=0.885, P=0.3878
	NEC	t=4.373***, P=0.0004	t=4.695***, P=0.0002
	LEC	t=1.675, P=0.1112	t=4.448***, P=0.0003
Ventral	WEC	t=0.4542, P=0.6551	t=1.607, P=0.1255
	NEC	t=1.863*, P=0.0789	t=2.195*, P=0.0415

LEC= Length of Epidermal cellsWEC= Width of Epidermal cellsNEC= Number of Epidermal cells
* Significant: t' value at 18 d.f. on 0.05% level is 1.734

The length of guard cells decreased significantly on dorsal surface of new leaves at polluted sites but the width of the same cells insignificantly increased at the same sites (Table 1 and 2). On ventral surface of leaves the length of guard cells increased significantly. However, this increase in width of the same cells was observed only in old leaves. There was reduction in width of cells of new leaves at pollutes sites. Similarly, the values of stomatal index and stomatal frequency were also observed to be higher for the leaves sampled from control site. Table (3) shows average number, length and width of epidermal cells of *Bougainvillea spectabilis*. The results indicated an increase number of epidermal cells on both surfaces of old and new leaves of polluted site as compared to control ones. This increase in number of epidermal cells was statistically significant, except for the leaves of ventral surface of new leaves (Table 4). Length and width of epidermal cells were increased in both new and old leaves on dorsal and ventral surface at polluted site as compared to control site, except on ventral surface of new leaves where a decrease in width of epidermal cells was registered. The length of cells was observed to be increased statistically significant on both surfaces of new and old leaves, except on ventral surface of new leaves (Table 4). Similarly, the width of epidermal cells was decreased insignificantly. The marked alterations in the size and number of foliar epidermal and guard cells, stomatal frequency and stomatal index in both dorsal abaxial and adaxial surface of plant species under this investigation are not unexpected. Several species investigating the effect of cement dust pollution on micro-morphological parameters of leaves of different plant species have reported similar results for Indian cities (Jacobson *et al.,* 1971 and Sai, 1986). The distorted shapes of stomata might have resulted due to lowering of $_{p}$H in the cytoplasm of guard cells and thus a change in turgour relations of stomatal complex (Kondo *et al.* 1980) and due to physiological injury within the leaf (Ashenden 1978).The decrease in number, length and width of stomata in the present study suggest that the species are more sensitive and does not have adoptive measures for mitigating the pollutants present in the air. However overall decrease in the number and size of stomata may be helpful in preventing the entry of pollutants into leaves, which can otherwise cause injury and death of the tissue of the leaves.

Conclusion

The present finding indicate that the exhaust released from automobiles and diesel engine vehicles causes abnormal changes in the foliar morphological characters of leaf of *Bougainvillea spectabilis*and these changes could be considered as bio-indicator of automobile pollution.

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