RESEARCH ARTICLE

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Effect of Traffic Exhaust on Anatomy and Micromorphology of *Nerium oleander* L. Leaves by Using Light and Scanning Electron Microscopy

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ABSTRACT: This study aimed to determine the effect of traffic exhaust on anatomical and micromorphological structures of *Nerium oleander* L. (Oleander) leaves. Oleander leaves were collected from two different sites; the urban and rural areas. The paraffin method using microtomes was applied for the cross-ections of leaves. To investigate the effect of traffic exhaust on the micromorphology of oleander leaves, the upper surfaces of leaves were photographed with SEM. The anatomical and micromorphological changes in leaves of the urban group showed that traffic exhaust pollution has significant effects on the anatomical and micromorphological structure of *N. oleander* L. leaves. In the urban area leaves surfaces were heavily loaded with pollution particles. The increase and decrease in measurements of several leaf anatomical tissues in the urban groups according to rural areas can be an answer to traffic exhaust stress. The detrimental effects of exhaust pollution were remarkably observed on epidermis and trichome cell structures in the leaves of oleander. Results obtained in this study clearly showed how the plants cope with traffic exhaust pollution stressed by anatomical changes.

Keywords: Anatomy, micromorphology, traffic exhaust, leaf, oleander

INTRODUCTION

Traffic exhaust has increased the level of metals in the environment and caused a detrimental effect on plant tissues (Aksoy and Sahln, 1999; Aksoy et al., 2000; Shafiq and Iqbal, 2007). Many studies show the harmful effects of pollutant gases (sulfur dioxide, nitrogen dioxide, and ozone) on plants (Bussotti and Ferretti, 2009; Calfapietra et al., 2009; Dineva, 2004).

Leaves are affected by auto-exhaust pollution a lot instead of stem and roots as well. Traffic exhaust gases have detrimental effects on plant structure and plant function. Therefore, many anatomical researches generally have been carried out on leaves subject to traffic exhaust pollution (Silva et al., 2005; Verma et al., 2006). The leaf can serve as a good bio-indicator for traffic exhaust. Higher plant leaves have been used as bio-indicator for heavy metal pollution since the 1950s. There is an increase in the use of higher plant leaves as bio-indicator for heavy metal pollution in the terrestrial environment (Dmuchowski and Bytnerowicz, 1995; Aksoy and Öztürk, 1996).

Oleander is greatly cultivated as an ornamental plant, but the plant has contained toxic compounds (cardiac glycosides and cardenolides) to man, animals, and certain insects (Soto-Blanco et al., 2006; Langford and Boor, 1996). Oleander needs virtually no water and has beautiful flowers. Because of this, oleander is one of the road site plants which is preferred mostly to be planted around the highways. Oleander is preferred as a possible bio-indicator to heavy metal pollution for several reasons in researches. The first reason is that the plant grows in both urban and rural areas. The second reason is the classification and cultivation of the oleander is easy and inexpensive. The last reason that is it has a wide ecological distribution. This plant is often used for heavy metal distribution in some countries as well (Seaward and Mashhour, 1991; Sawidis et al., 1995). The aim of the study was to examine the effect of traffic exhaust on leaf anatomy and micromorphology of *Nerium oleander* L. leaves.

MATERIALS AND METHOD

In this study, leaves of *Nerium oleander* L. (Oleander) were used as research material. The experiment was carried out in the Botanical Laboratory of Biology Department, Faculty of Art and Science, Amasya University. The study was carried out by using leaf samples collected from oleander plants in two different sites: the urban and rural areas. The urban site was Bornova Evka 3 roadside, an area with a distance of about 15 km away from İzmir, a megacity in western Turkey with a population of 4.28 million. The rural site was Urla Karapınar neighborhood, an area about 55 km away from İzmir center.

The leaf samples were collected from 10 oleander plants in each area. To reflect all the canopy leaf samples were collected from the bottom, middle, and top of the canopy. Leaf samples were fixed in 70% ethyl alcohol for the width and length measurements of various leaf tissues. The studies were performed on average 30 fresh specimens kept in 70 % alcohol The

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paraffin method using microtomes was applied for the cross-sections of the leaf (Algan, 1981). ‘Safranin O’ and ‘Fast green’ was used for dying. A Leica ICC50 HD binocular light microscope, a Leica Digital Camera, and X10 - X40 objectives were used to measure the width-length of various tissues in leaf. Anatomical measurements have been based on 30 cells. Preparations were photographed with a Leica ICC50 HD binocular light microscope and a Leica Digital Camera.

To investigate the effect of exhaust pollution on the micromorphology of oleander leaves, the upper and lower surfaces of leaves collected from urban and rural areas were also photographed with the scanning electron microscope (SEM 515, Philips, The Netherlands). The specimens were sputtered with gold in a vacuum coating unit (SC 500, Emscope, Ashford, United Kingdom) (Robinson et al., 1987).

SPSS 20.0 for Windows was used for statistical analysis. The variance analyses were made by using the Tukey test (Tukey, 1954).

RESULTS AND DISCUSSION

The values of width-length measurements of leaf anatomical layers in transverse sections belonging to each groups were given in Table 1. All results except for length measurements of palisade parenchyma and total spongy parenchyma in width-length measurements of leaf anatomical layers were lower in urban sites than in rural areas (Table 1, Figure 1 a,b,c,d,e,f). Such a decrease in width-length measurements of leaf anatomical layers seen in urban areas was generally statistically significant as compared to the rural area except for total palisade parenchyma. According to the table, the length measurement of palisade parenchyma was higher in an urban area than in a rural site. Total palisade area was lower whereas total spongy parenchyma area was higher in an urban area when compared to the rural area, but this decrease and increase weren’t statistically significant (Table 1). Besides, the values of width-length measurements of the midrib trachea were lower in an urban area than in a rural area and the least thickness of the midrib trachea was determined in the urban site.

Plants generally tolerate traffic exhaust pollution stress with several morphological and anatomical responses that affect the size of stomata and epidermal cells and also the stomata index (Raina and Bala, 2011; Tiwari, 2012). Therefore, lots of plants are used as a bio-indicator for traffic exhaust pollution because of the higher sensitivity to several pollutants related to traffic. In the present study, all results except for length measurement of palisade parenchyma in the urban group were lower in an urban area than in a rural area, whereas the length measurement of palisade parenchyma was higher in urban site than in rural area. A similar reduction in several anatomical tissues of leaves at polluted areas was found in several studies as well (Saadabi, 2011; Shrivastava and Mishra, 2018). According to Salgare and Thorat (1990), the reduction mentioned above could be an adaptation to vehicle exhaust pollution to reduce accumulated metals caused by traffic-based pollutants. This adaptation can help plants minimize stress and maximize the use of internal and external resources (Dineva, 2004). Similarly, in the present study, the width measurement of palisade parenchyma was lower in the urban group than in rural areas. The decrease in the width measurement may be the response to heavy metals arise from traffic exhaust and can be an adaptation to reduce accumulated metals caused by traffic-base pollution. These results are parallel with another researcher mentioned above. Thus, Sridhar et al. (2011) showed that Cr accumulation caused a decrease in leaf thickness and shrinkage of epidermal, spongy, and palisade parenchyma cells in *Pteris vittata* L. However, the length thickness of palisade parenchyma was higher in urban areas than in rural area. This increase in the length thickness of palisade parenchyma can be raised from the damaged hormonal balance in the leaves because of traffic exhaust gases. Similarly, Gomes et al. (2011) suggested that heavy metal may change the phytohormonal balance in several plant organs. In another study, it was stated that Ag and Cu metals had widened intercellular spaced in the parenchyma cells of *Potamogeton* L. species (Al-Saadi et al., 2013).

When it comes to the effect of traffic exhaust pollution on micromorphology of upper surfaces in oleander leaves, it was seen that this kind of pollution decreased the amount of trichome density in the urban area, particularly the traffic exhaust caused deformation in trichome structure (Figure 2 a,b,c,e,f). However, the leaf surface is covered with lots of nondamaged trichomes in the rural area (Figure 2 a,b,c). There was a serious decrease in the amount of trichome density on the upper surface of leaves taken from the urban group. Besides, structural deformations and breakdown in trichomes belonging to the urban area have been observed as well (Figure 2 e,f). The same deformation in upper epidermis cells was seen in urban group. The wrinkles, disintegrations in upper epidermis cells were also observed in urban sites (Figure 2 e,g). But, the leaf surface is covered with nondamaged epidermis structures in the rural area than in the urban group (Figure 2 d). There wasn’t observed any pollution particles on the upper surface of the leaf in the rural area, whereas lots of pollution particles were seen on the surface of the leaf in the urban area (Figure 2 d, h). It was also seen that there was no cohesiveness in the trichome structure present on the stomatal chamber in the lower surface of the leaf in a rural area, but the cohesiveness was seen in the trichome present on the stomatal chamber in the lower surface of the leaf in the urban area (Figure 3 a, b).

Several studies carried out on the toxic effects of heavy metals showed that these metals damaged epidermis cells, collapsed trichomes, and root hairs (Maleci et al., 2001; Mangabeira et al., 2001). In the present study, there was an increase in the number of damaged trichomes and epidermis cells in the urban group than in rural areas. It was clearly seen that such a pollution caused deformation in trichome structure and epidermis cells in the oleander leaves. The results in the paper are in agreement with the data found by the researchers mentioned above. The leaf surfaces were also covered with pollution particles in the urban group. Particularly, in the urban area, the leaf surfaces were full of these particles.

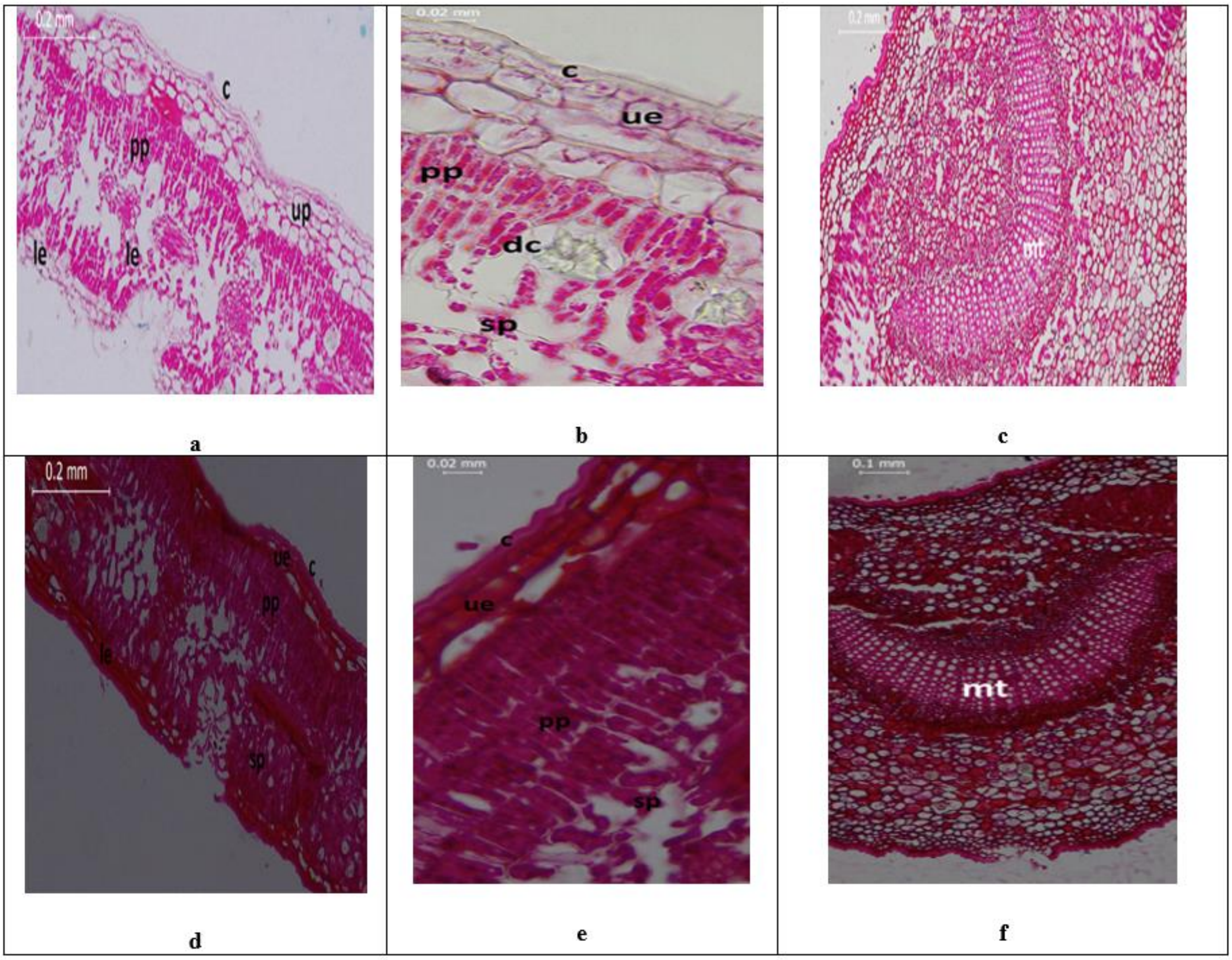

Figure 1. *Light microscopy photographs show transverse sections of leaves in rural area (a,b,c) and in urban area (d,e,f) c: cuticle, ue: upper epidermis, pp: palisade parenchyma, sp: spongy parenchyma, le: lower epidermis, mt: midrib trachea, dc: druse crystal.*

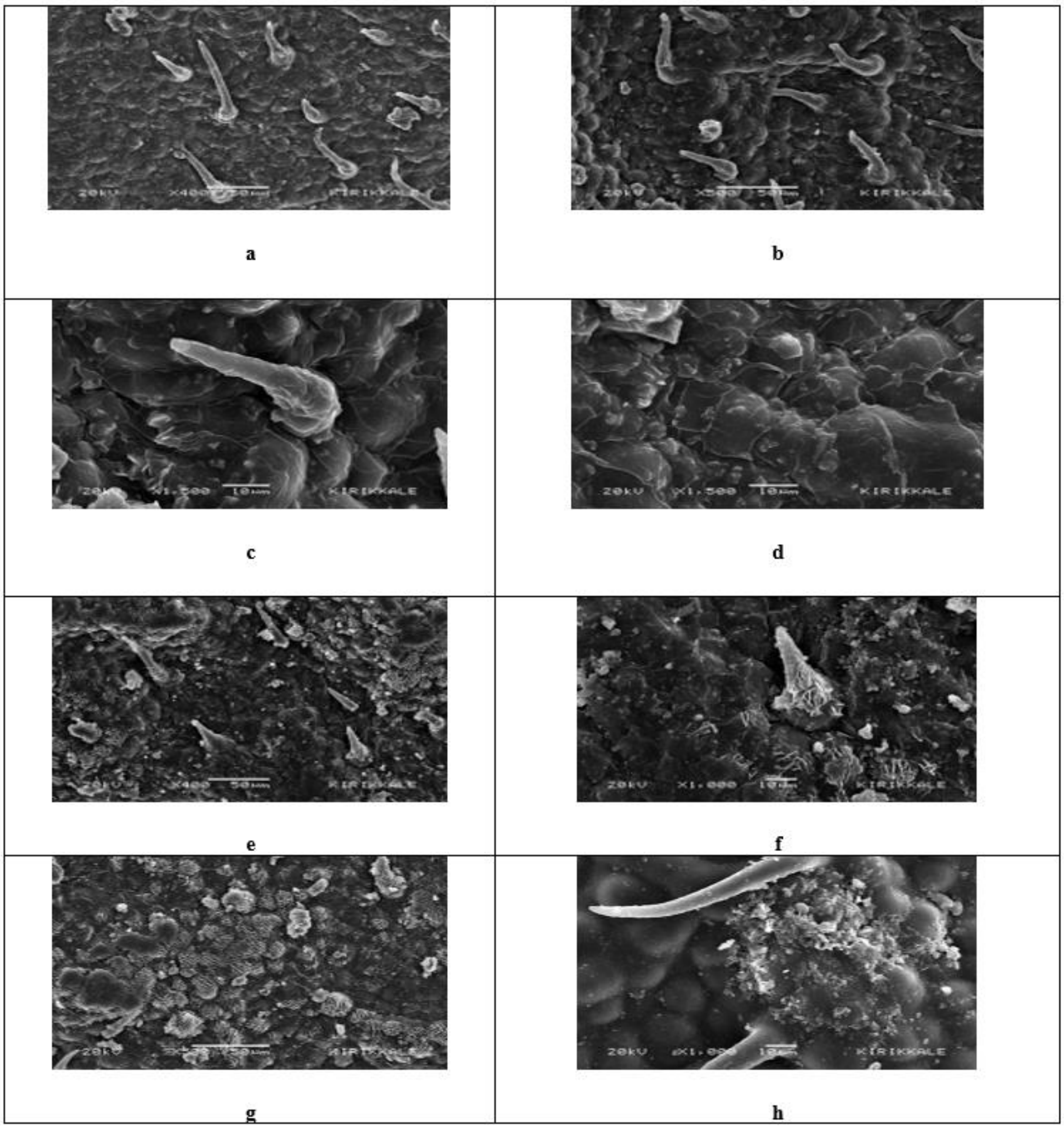

Figure 2. *SEM photographs show upper leaf surface of oleander leaves. Nondamaged trichome density in rural area (a), Nondamaged trichome density in rural area (b), Nondamaged trichome structure in rural araea (c), Nondamaged epidermis structure in rural area (d), Structural deformation in trichomes and epidermis in urban area (e), Damaged trichome structure in urban area (f), Damaged epidermis structure in urban area (g), Pollution particules on the surface of leaf in urban area (h).*

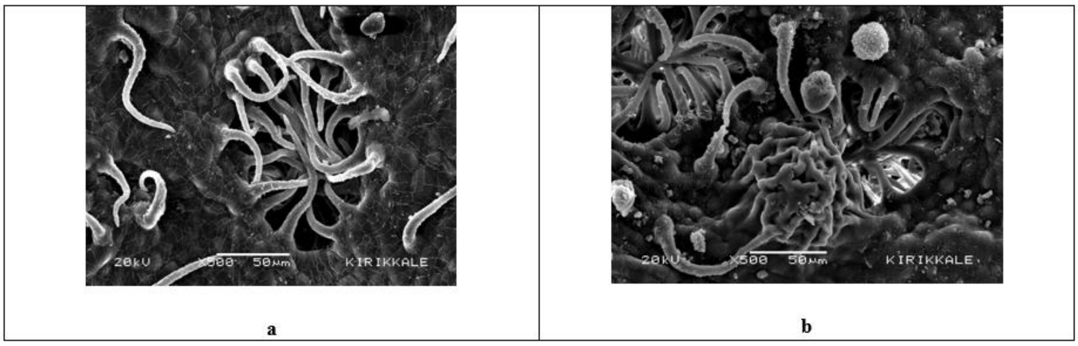

Figure 2. *SEM photographs show lower leaf surface of oleander leaves. No cohesiveness in the trichome structure present on stomatal chamber in the lower surface of leaf in rural area (a), The cohesiveness in the trichome structure present on stomatal chamber in the lower surface of leaf in urban area (b).*

Table 1. *The values of width-length measurements (µm) of leaf anatomical layers in transverse sections belong to each groups*

Anatomical Layers		Group	
		Rural area	Urban area
Cuticle		22.833 ± 0.675 [b]	12.083 ± 0.319 [a]
Upper Epidermis	Width	27.917 ± 1.403 [b]	18.750 ± 0.696 [a]
	Length	16.500 ± 0.488 [b]	11.917 ± 0.332 [a]
Palisade Parenchyma	Width	10.083 ± 0.305 [b]	8.333 ± 0.276 [a]
	Length	50.000 ± 2.500 [b]	116.083 ± 3.389 [a]
Total Palisade Parenchyma		118.000 ± 1.709	117.583 ± 2.837
Total Spongy Parenchyma		170.583 ± 2.983	179.000 ± 4.182
Midrib Trachea	Width	14.167 ± 0.527 [b]	9.500 ± 0.303 [a]
	Length	14.250 ± 0.342 [b]	9.167 ± 0.301 [a]

The tables show that the differences among ‘a’ and rural area, ‘b’ and urban area are statistically significant ($p<0.05$)

CONCLUSION

In conclusion, the anatomical and micromorphological changes in leaves of the urban group showed that auto-exhaust pollution has a significant effect on the anatomical and micromorphological structure of *N. oleander* L. leaves. The increase and decrease in measurements of several leaf anatomical tissues in the urban group according to rural area can be an answer to traffic exhaust pollution. The plant grown under traffic conditions may overcome heavy metal pollution based on traffic exhaust showing some anatomical changes. The detrimental effects of traffic exhaust pollution were clearly seen on epidermis and trichome cell structures in the leaves of oleander. In conclusion, results obtained in this study clearly showed how the plants cope with traffic exhaust pollution stressed.

CONFLICT OF INTEREST

No conflict of interest was declared by the authors.

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