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ORIGINAL ARTICLE

A COMPARATIVE STUDY OF STOMATAL AND EPIDERMAL CELL CHARACTERS OF SOME RUBIACEAEOUS SPECIES GROWING IN INDUSTRIAL AND VILLAGE AREAS OF HOWRAH DISTRICT, INDIA

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***Abstract**: Stomata and epidermal cells are present on the leaf surface and are often directly exposed to the pollutants present in the environment. There are several reports about the changes in the parameters related to stomata and epidermal cells due to environmental pollution. Keeping this in view, a study was conducted on the leaf anatomical characters in some commonly growing Rubiaceae members from the industrial city of Howrah, which was compared with the leaf samples of same species growing in less polluted village area of Gangadharpur, Howrah district, West Bengal. It was found that, the density (number per mm^2 leaf area) of stomata and epidermal cells were increased in polluted area. Oldenlandia corymbosa showed the highest level of increase in stomatal count (32.75%) followed by Ixora coccinea (31.06%), Neolamarckia cadamba (26.66%) and Gardenia jasminoides (16.90%). For epidermal cells also, similar pattern was observed. The stomatal index showed the tendency of decrease in polluted environment, which was very little (0.02%) for Ixora coccinea, 1.5% in Gardenia jasminoides, gradually increased to 4.5% in Oldenlandia corymbosa, which reached the maxima of 13.96% in case of Neolamarckia cadamba. Maximum stomatal clogging was found in Oldenlandia corymbosa (16%), which was 15% in I. coccinea, 8% in N. cadamba and 7% in G. jasminoides, growing in polluted environment. In less polluted area, clogging percentage was found to be <2%. Regarding the size of stomatal opening, the length has been decreased up to 33.3% in O. corymbosa. When the breadth of aperture was considered, the decrease was highest in I. coccinea and O. corymbosa, which was 50%, followed by G. jasminoides (37%) and N. cadamba (32%). The study reveals that characters of stomata and epidermal cells are strong indicators of the presence of pollutants in the environment.*

Key words: Stomata, epidermal cell, stomatal index, stomatal opening, Rubiaceae, pollution.

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1. INTRODUCTION

When any kind of undesirable effect is caused by the presence of any substance and/or heat in the environmental media (water, air, land), it is termed as pollution [1]. Pollution is a threatening problem for living organisms throughout the globe. Air pollution is a major environmental issue, mainly in the developing countries [2]. Plants are the primary producer, capable to convert the solar energy to chemical energy by photosynthesis, are found to be sensitive to the contaminants in atmosphere [3-6]. Leaves, the site of photosynthesis, when exposed to atmospheric pollutants are affected adversely [7]. There are reports of distinct changes in the leaf variables in the plants, growing in polluted areas, when compared to less polluted zones [8, 9] and hence leaves are often considered as bioindicator of environment [10]. Stomata are found to be present mostly on the leaf surfaces and directly exposed to all the pollutants present in the atmosphere. These are composed from a pair of bean shaped guard cells joined at their ends, encircling an aperture or pore. Stomatal apertures are the sites of exchange of water, carbon dioxide and oxygen in plant [11]. Previous studies indicated that the parameters related to stomata and epidermal cells are determined by existing environmental conditions [12, 13].

The city of Howrah, located on the western flank of the river Hooghly, opposite to the city of Kolkata, is an industrial centre of eastern India with high level of atmospheric pollutants. In th year 2021, the ambient particulate matter, PM_{10} concentration in Howrah city was reported to be the highest (125 mg/m_3) in West Bengal [14]. The present study was conducted to make a comparative account of the stomatal and epidermal characteristics on the leaves of the commonly growing plant members of Rubiaceae in the industrial belt of Howrah city and a less polluted village area. Here, four species of Rubiaceae family were selected, which are commonly growing as an integral part of the local vegetation and ornamental plants.

2. MATERIAL AND METHOD

Collection sites

Mature leaves were collected from less polluted area of Gangadharpur village (site I, 22.586° N 88.159° E), and the highly polluted industrial area of Howrah city (site II, 22.580° N 88.329° E), having a distance of 19.5 km from Gangadharpur (Figure 1). In the collection day of 28th April, 2024, the Air Quality Index (AQI) of Howrah city was 108, which was unhealthy for sensitive groups" level [15].

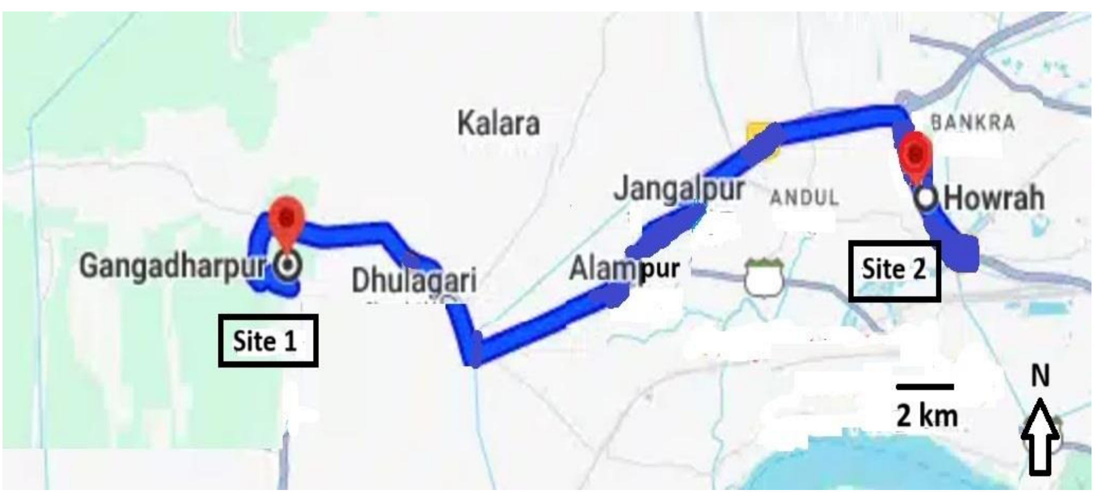

Figure 1. Location map showing site 1 (less polluted area of Gangadharpur village) and site 2 (highly polluted industrial area of Howrah city) in the state of West Bengal, India.

Leaf samples

Mature leaves were collected from the following plant species of Rubiaceae family characterized with paracytic stomata (Figure 2):

1. *Gardenia jasminoides* J. Ellis (perennial ornamental woody shrub)
2. *Ixora coccinea* L. (perennial ornamental shrub)
3. *Neolamarckia cadamba* (Roxb.) Bosser [Synonym: *Anthocephalus cadamba* (Roxb.) Miq., perennial tree],
4. *Oldenlandia corymbosa* L. (annual herb)

All these four plants were found growing in the village of Gangadharpur (site I) and city of Howrah (site II). Leaves were collected in the last week of April 2024, to avoid seasonal variation. The mature leaf samples were collected, then washed with running water, to remove the dust and debris before drying in air.

Anatomical Studies

Micromorphometric studies for stomatal density, epidermal cell density, stomatal index and clogged stomata were carried out in 10 randomly selected microscopic fields in the epidermal peels of the lower or abaxial leaf surface for each leaf specimen to get the average value. The area of microscopic field, cells and apertures was measured by using ocular micrometer (inserted in the eye piece). The exact measurement values were calculated by multiplying with ocular constant, which was derived by the comparison between ocular and stage micrometer under the objective of the compound microscope (Lawrence and Mayo).

a. Determination of stomatal and epidermal cell density

Stomatal and epidermal cell density was determined as the average number of stomata and epidermal cells per square millimetre of the lower epidermis of the leaf.

b. Determination of stomatal index

Stomatal index was calculated by using the formula [16]:
Stomatal index (%) = (S/S+E) × 100 where, S and E are the number of stomata and epidermal cells per unit area respectively in the respective microscopic view field.

c. Determination of clogged stomata percentage

The percentage of clogged stomata (due to particle deposition) with respect to total stomatal count in the microscopic field (10 observations) was calculated for each leaf sample.

d. Determination of the length and breadth of stomatal opening

Length and breadth of stomatal opening at the lower epidermis of the leaves were determined under compound microscope using the ocular micrometer.

3. RESULTS AND DISCUSSION

The abaxial foliar epidermal character of the studied leaf samples elicited the presence of mostly paracytic type of stomata in all the four members of Rubiaceae, namely *Gardenia jasminoides*, *Ixora coccinea*, *Neolamarckia cadamba* and *Oldenlandia corymbosa* (Figure 2).
In the present study, it was found that the stomatal density (number of stomata per mm^2 leaf epidermal area) is higher in the polluted area (site 2), compared to less polluted area (site 1) for all of the leaf specimen.

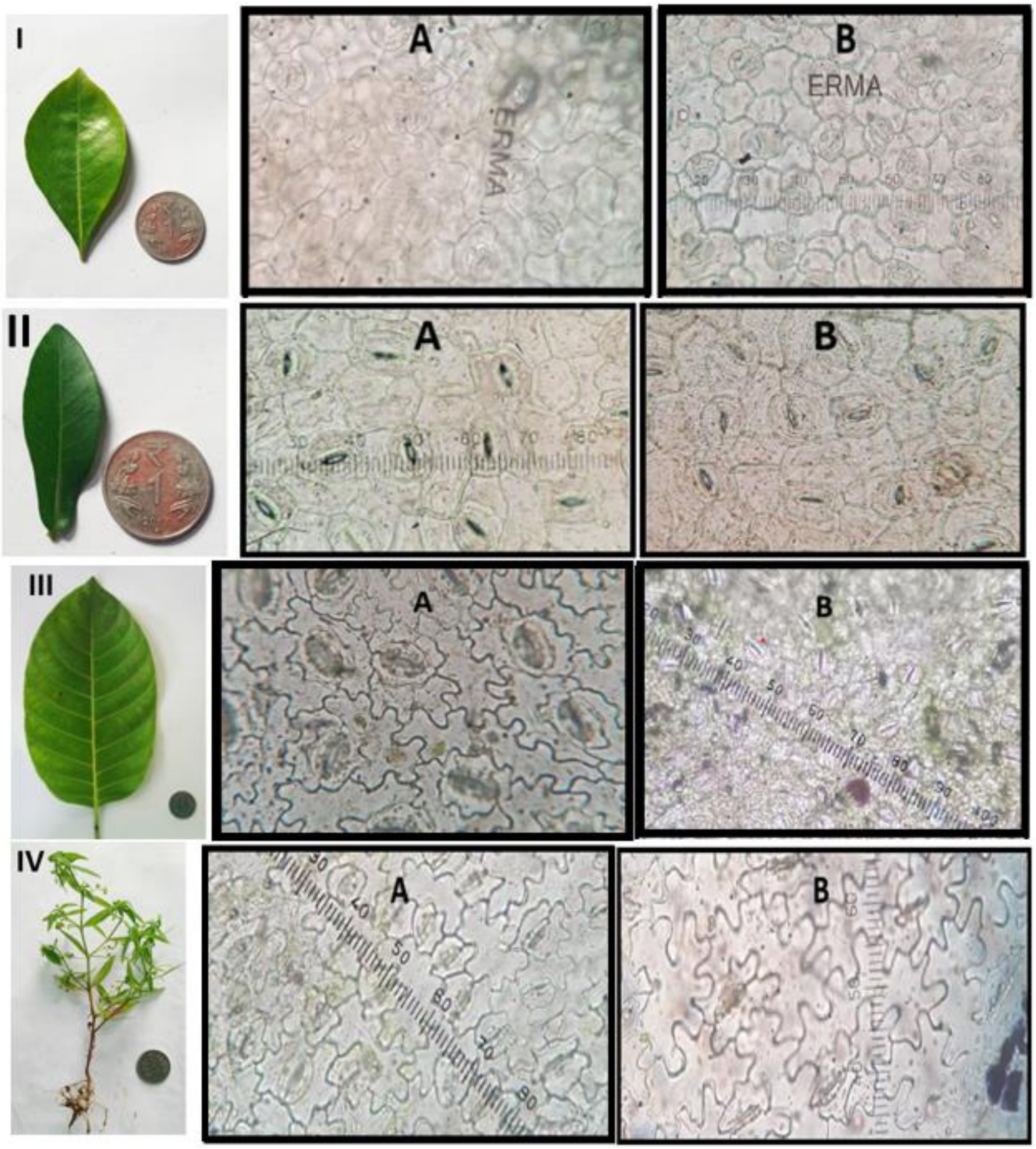

Figure 2. Leaf specimen (left) and epidermal microscopic view of the epidermal peel of the abaxial surface showing the stomata and epidermal cells for the leaves of (I) *Gardenia jasminoides*, (II) *Ixora coccinea*, (III) *Neolamarckia cadamba* and (IV) *Oldenlandia corymbosa* in less polluted (A -Site 1) and polluted (B - Site 2) area.

Oldenlandia corymbosa showed the highest level of increase in stomatal density (32.75%) followed by *Ixora coccinea* (31.06%), *Neolamarckia cadamba* (26.66%) and *Gardenia jasminoides* (16.90%) respectively [Figure 4]. Ekpemerechi *et* al. [17] also reported increase of stomatal number in polluted environment for the members of Euphorbiaceae from Nigeria. Devkota and Jha [18] reported the rise of stomatal density with high pollution level. However, on the contrary, in case of the leaves of plane tree from Iran, the stomatal density was reported to be lower in urban area [19].

In case of epidermal cell also, the similar pattern was observed. When epidermal cell density is considered, *Neolamarckia cadamba* showed the highest level of increase (53.85%), followed by *Gardenia jasminoides* (35.27%), *Ixora coccinea* (31.35%) and *Oldenlandia corymbosa* (10.52%) respectively (Figure 3).

This observation corroborates with the report on the common plant species growing in Kathmandu valley of Nepal [18].

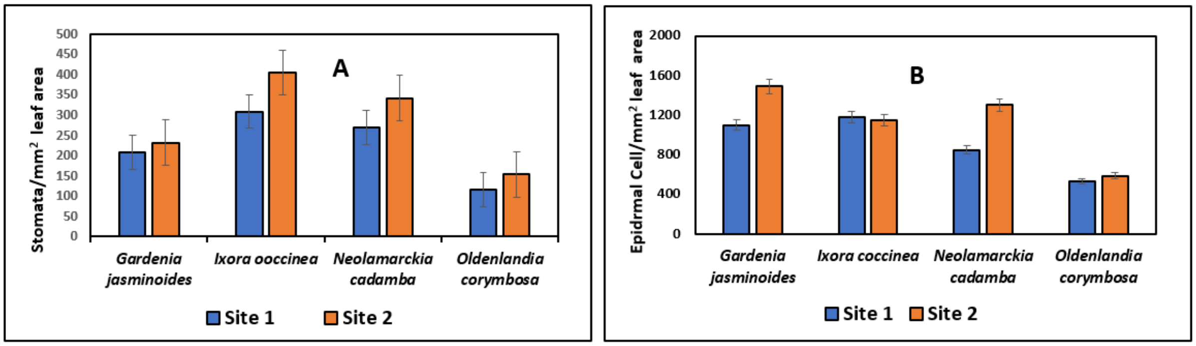

Figure 3. Average stomatal (A) and epidermal cell (B) density (no./mm^2 area) in the lower epidermis of the leaves of *Gardenia jasminoides*, *Ixora coccinea, Neolamarckia cadamba* and *Oldenlandia corymbosa* in less polluted (Site 1) and polluted (Site 2) area. Bar indicate standard deviation ($p<0.005$).

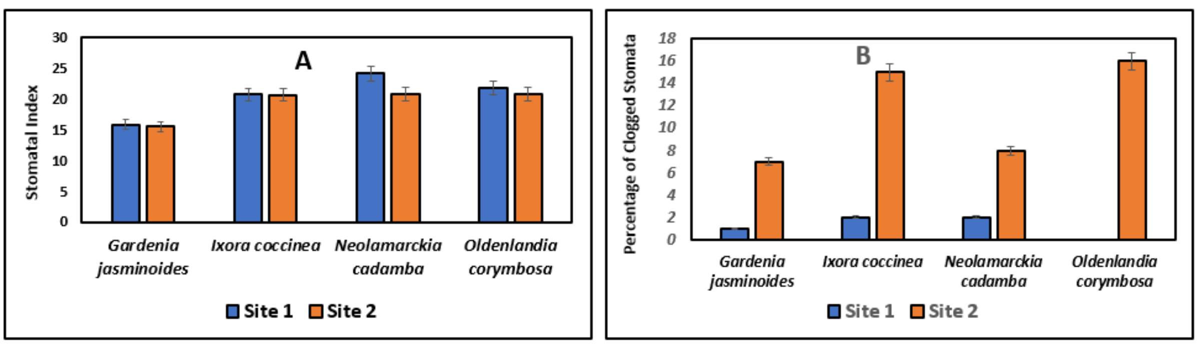

Figure 4. Stomatal index (A) and the average percentage of clogged stomata (B) observed in the lower epidermis of the leaves of *Gardenia jasminoides*, *Ixora coccinea, Neolamarckia cadamba* and *Oldenlandia corymbosa* in less polluted (Site 1) and polluted (Site 2) area. Bar indicate standard deviation ($p<0.005$).

The number of stomatal openings and the epidermal cells are found to vary depending of species, maturity level of the leaves, climatic factors, environment in which the plant is grown and several other factors [20]. In this aspect, stomatal index has been noticed as a more or less constant factor, which is not influenced by age, climate and environmental factors related to the plants [21].

In the present study, stomatal index [Figure 4A] in *Gardenia jasminoides* ranges between 15.59 – 15.83, which falls between the reported range of 14.66 – 25.61 [21, 22].

When Patil and Patil [23] investigated the foliar epidermal characteristics on the Rubiaceae plant members, the stomatal index of *Gardenia jasminoides* was found to be 23.80 (much higher than the value compared to the present study). In the same report, stomatal index of *Ixora* ranged between 16.55-20.58, nearly corroborative with the present value 20.71-20.75. Here the stomatal index showed the tendency of decrease in polluted environment, which was very little (0.02%) for *Ixora coccinea*, 1.5% in *Gardenia jasminoides*, then gradually increased to 4.5% in case of *Oldenlandia corymbosa*, which reached the maxima of 13.96% in case of *Neolamarckia cadamba*.

In the leaf samples studied, a number of stomata were found to be clogged, when the epidermal peels were observed under microscope. The percentage of clogged stomata (Figure 4B) were more in polluted area (Site 2) compared to less polluted are (Site 1). Maximum stomatal clogging was found in *Oldenlandia corymbosa* (16%), which was 15% in *Ixora coccinea*, 8% in *Neolamarckia cadamba*, and 7% in *Gardenia jasminoides*, growing in the polluted city area. In less polluted area, *Oldenlandia corymbosa* showed no stomatal clogging, whereas it was around just 1-2% in other three Rubiaceae species.

Similar observation was reported by Devkota *et al*. for the plants growing in Kathmandu valley area of Nepal [18]. In a study focussed on the effects of air pollution on the leaf structure of some Fabaceae species, deposition of dark phenolic deposits was reported within palisade and spongy parenchyma [24]. Such physical damage and blocking of gaseous exchange and transpiration occurs, basically due to particulate matter and dust deposition and coating on the leaf surface. In the leaves of *Quercus petraea*, diffusion resistance has been reported to be interfered by particle deposition, leading into stomatal closure [25]. In this way, particle deposition is a damaging factor in the physiological condition of plants.

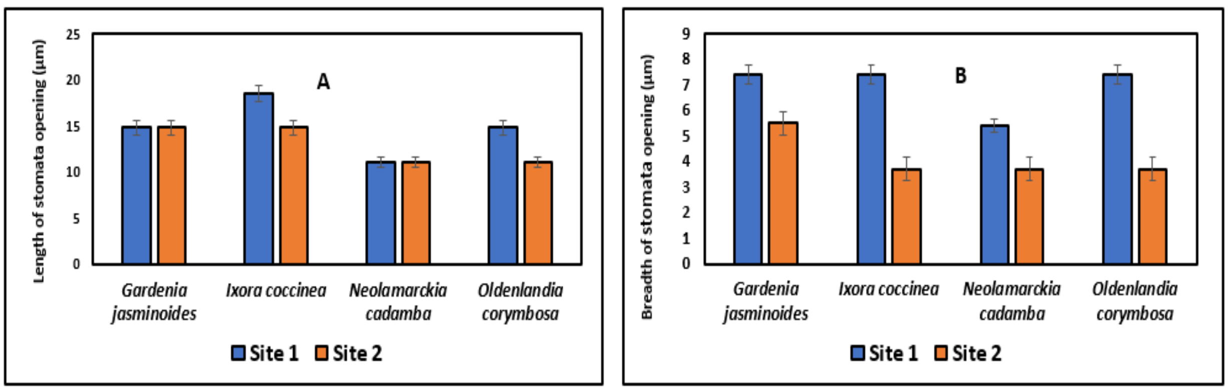

Figure 5. Average length (A) and breadth (B) of stomatal opening observed in the lower epidermis of the leaves of *Gardenia jasminoides*, *Ixora coccinea*, *Neolamarckia cadamba*, and *Oldenlandia corymbosa* in less polluted (Site 1) and polluted (Site 2) area. Bar indicate standard deviation (p<0.005).

Size of stomatal aperture is very important, as it is the site for transpiration and gaseous exchange in plants. In our study, the length of stomatal opening elicited no change in case of G*ardenia jasminoides and Neolamarckia cadamba* and showed 25-33.3% decrease for *Ixora coccinea* and *Oldenlandia corymbosa* (Figure 5A).

However, in case of the breadth of stomatal aperture from the leaves collected from polluted area, there was distinct level of decrease. For *Ixora* and *Oldenlandia*, there was 50% decrease in the breadth of stomatal aperture, which was 37% for *Gardenia* and 32% for *Neolamarckia cadamba* (Figure 5B). The observation presented here is supported by the reports by Ekpemerechi *et al.* [17] and Tiwari *et al.* [26], where workers found that the size of stomatal opening decreases significantly (p<0.05) from rural to urban area.

4. CONCLUSION

In this way, the present study indicates that exposure to polluted environment causes distinct changes in the stomatal and epidermal characters of the leaves of the local plant members of same species, when compared to these growing in less polluted area. This preliminary work establishes the importance of stomatal and epidermal cell characters as the markers of plant's response to environmental pollution, and needs further in-depth analyses.

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