Supported by tetfund

EFFECT OF SIMULATED ACID RAIN ON MORPHOLOGY AND STOMATAL TRAITS IN LEAVES OF *Celosia argentea* L. (AMARANTHACEAE) AND *Corchorus olitorius* L. (MALVACEAE)

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Received: May 19, 2021 **Accepted:** July 18, 2021

Abstract: The impact of acid rain, resulting from the industrial emissions on plants, is of global concern. This study investigated the effect of Simulated Acid Rain (SAR) on some quantitative leaf morphological characters and stomatal traits of *Celosia argentea* and *Corchorus olitorius* which are both cultivated for their edible succulent leaves, using standard established procedures. Young seedlings of the two plant species were exposed to SAR condition of pH 2.0 while control plants were exposed to distilled water (pH 7.0) for a period of five weeks. A marginal increase in morphological characters namely plant height, leaf numbers, leaf area and petiole length was observed in SAR-treated *C. olitorius* when compared with the control plants. However, reverse is the case in *C. argentea* where all the characters were significantly reduced ($p<0.05$) except petiole length which gave a marginal increase in SAR-treated plants. Stomatal traits were reduced in the two species except on the adaxial surfaces of *C. argentea* leaves. Overall, the possible mechanism by which *C. olitorius* respond to SAR is through reduction in stomatal index and area of guard cells on the adaxial surfaces of the leaf, both of which consequently slow down the rate at which water and energy are lost through transpiration.

Keywords: *Celosia argentea*, *Corchorus olitorius*, simulated acid rain, stomatal index

Introduction

In developing countries such as Nigeria, urban air pollution is a major environmental problem (Mage *et al.*, 1996; Saheed *et al.*, 2017). Acid rain is a product of precipitation that has been acidified when pollutants such as oxides of sulfur and nitrogen react with atmospheric moisture (Kita *et al.*, 2004). This deposition, usually in form of rainwater, has pH of 3.0 or more and capable of causing significant damage to plants as well as the entire ecosystem (Sant'Anna-Santos *et al.*, 2006).

In Nigeria, especially in the Niger Delta, acidic rainwater with pH range of 4.71 – 5.73 was reported by Nduka *et al.* (2008). Their long term studies raised concerns whether organisms, most especially plants, are safe in Nigeria's Niger Delta environment, an area already noted with widespread issues of oil pollution and its effects on plant growth and development. Interestingly, other regions of the country are not left out as enunciated by Odiyi and Eniola (2015). These are pointers that many areas are potentially vulnerable to the menace of acid rain deposition as a result of increase in the consumption of fossil fuels and widespread industrial activities such as metal smelting which is rampant in many parts of Nigeria where old metallic items are recycled.

Exposure of plants to acid rain results in myriads of characteristic symptoms of foliar as well as modified leaf anatomy and morphology (Stoynora and Velikova, 1998; Park and Yanai, 2009). These are of utmost consideration in the cultivation of leafy vegetables, such as the two – *Celosia argentea* and *Corchorus olitorius*, investigated in the present study.

Celosia argentea L. (Kuntzie) (Amaranthaceae), the common Cockscomb, is a short-lived, annual, erect herb that usually reaches up to 1 m in height. It originates from tropical Africa but the plant has spread wherever humans have gone in the tropics and subtropics, both as a weed and as an escape from cultivation. Although it is a plant of the tropics, it is also grown in the subtropics and warm temperate zones. It is propagated by seeds. It grows best in areas where annual daytime temperatures are within the range 25 - 30°C. It prefers a mean annual rainfall in the range 1,500 – 2,500 mm. For best leaf production for which it is sought, the plant requires a fertile, moisture-retentive but well-drained soil although it is tolerant of a range of soil types. Its early vegetative growth is rather slow, but flowering may occur 6 - 7 weeks after sowing. The leaves of the plant are arranged alternately and light green in colour but darker on flowering shoots. The plant is characterized by its brightly coloured flowers which may be red or purple. It is commonly cultivated as an important and nutritious leaf vegetable. The leaves and young shoots are cooked and used in soups and stews. It is generally used in ethnomedicine to treat diarrhoea, hypertension and poison from snake bites.

Corchorus olitorius L. (Malvaceae) is widely known as Jute mallow or Jew's mallow or West African sorrel, among many other local names. It is propagated by seeds. The leaves and young fruits are used as a leaf vegetable while the dried leaves are used for tea and as a soup thickener. *C. olitorius* is an erect herbaceous plant, fairly branched and often grows about 1.5 m high. The taproot leads to a sturdy and hairless stem, which is green and which sometimes turns a little woody on ground level. It grows best in a fertile, moisture-retentive but well-drained soil. The serrate acute leaves, often picked as vegetables, are alternate and are 6 to 10 cm long and 2 to 4 cm wide. The plant carries yellow flowers in solitary or two-flowered cymes. The fruit is spindle-shaped, dehiscent and divided into transversal sections through five valves with seed chambers. Every seed chamber contains 25 to 40 seeds, which can sum up to 125 to 200 seeds per fruit.

Acid rain also poses more serious damage to plants in any ecosystem, when compared to other organisms, largely as autothrophs requiring carbon dioxide obtained from the atmosphere as well as water and mineral nutrients absorbed from the soil in order to produce food for other organisms. Apart from the fact that it causes both physical and internal damages to the leaves, it also rids the soil of very important nutrients utilized by plants such as magnesium and calcium, which in turn increase the vulnerability of plant to pests and drought (Mello and Almeida, 2004; Kumaravelu and Ramanujam, 2008).

Studies based on greenhouse experiments have investigated the effect of SAR on plants (Nouchi, 1992; Temple *et al.*, 1992; Gabara *et al.*, 2003; Silva *et al.*, 2005; Silva *et al.*, 2006; Sant'Anna-Santos *et al.*, 2006; Kumaravelu and

Ramanujam, 2008; Lal, 2016). Majority of these studies documented deleterious effects of SAR on plant biomass as well as morphometric parameters such as leaf area and leaf length. Visible injuries on the leaf surfaces as a result of impairment and distortion of the anatomical structure of the leaf have also been reported (Temple *et al.*, 1992; Silva *et al.*, 2005; Sant'Anna-Santos *et al.*, 2006; Kumaravelu and Ramanujam, 2008; Lal, 2016). Despite the fact that acid rain is evident in Nigeria, very few studies such as Odiyi and Eniola (2015) and Macaulay and Enahoro (2015) have investigated effect SAR on plants in general and leafy vegetables in particular. Their studies reported reduction in leaf biomass and morphological changes such as chlorosis, necrosis, and leaf abscission among others, in leaves of cowpea and maize, both which are not leafy vegetables.

Information on changes to stomatal features consequent to SAR and responses by the plants in form of morphologic changes will go a long way to unravel the mechanism underlining the overall responses of these plants to the menace of acid rain deposition. Premised on the importance of *C. argentea* and *C. olitorius* as leaf vegetables in the diets of Nigerians, their widespread and large-scale cultivation in various parts of the country and the increasing rate of industrialization which had resulted in acid rain pollution over the years, this study seeks to investigate their responses in forms of leaf stomatal traits and morphology to a regime of SAR conditions.

Material and Methods

Study site and seeds collection

The study was carried out under greenhouse conditions at the Department of Botany, Obafemi Awolowo University, Ile-Ife, Osun State, Nigeria. Disease-free seeds of *C. argentea* and *C. olitorius* were collected from the National Horticultural Research Institute (NIHORT), Ibadan, Nigeria.

Establishment of test plants

Seeds of the two species of test plants were sown by broadcasting them in two separate wide wooden trays filled with top soil fortified with farmyard manure as supplement. Emerging seedlings were sparingly watered twice daily to saturation levels of the soil substrates. The emergence of seedlings occurred in the third week of germination. Seedlings of the two species were transplanted into ten polythene planting bags each with one seedling per pot. Subsequently, they were separated into two experimental groups – control and those subjected to SAR treatments.

Preparation of simulated acid rain condition

SAR condition was prepared following the method of Odiyi and Bamidele (2014). Concentrated sulphuric acid (H_2SO_4) and concentrated nitric acid (HNO_3) were mixed in ratio 2:1. The resulting solution was then diluted with distilled water and made to pH 2.0. Distilled water of pH 7.0 was used for control experiment. Each treatment had five replicates arranged in a completely randomized design. The application of the SAR treatments began one week after transplanting when seedlings of selected test plants had become fully established. Both treated and control plants were sprayed with 10 mL of SAR solution and distilled water respectively every three days, using a medium-size pressurized sprayer on the plants following the method of Evans *et al.* (1981). The plants were grown for five weeks before the termination of the study.

Morphological studies

Data of quantitative morphological characters such as plant height, leaf area, petiole length and number of leaves were recorded at 21 days after commencement of treatments on both the control and the experimental groups. Plant height and petiole length were measured using hand-held meter rule. Petiole length was measured from the basal end of the leaf to the area of attachment to the stem (Pandey and Singh, 2011). Leaf area was determined using Leaf Area Meter (Portable Laser CI-202; CID – Bio-Science). Areas for three leaves per pot for each of the ten plants were measured non-destructively using the leaf area meter while they were still attached to the plant body. Mean value for three leaves per plant was determined and used to form the ten replicates for each of the treatment and control.

Studies of leaf epidermal surface

To study the stomatal traits, consisting of stomatal index and guard cell area, sizeable portions of mature leaves of *C. argentea* and *C. olitorius* for both the control and treated plants were cut from standard median portion of the mature and well-expanded leaves. Epidermal peels for both adaxial and abaxial surfaces were obtained using the protocol of Saheed and Illoh (2010) and immediately preserved in 50% ethanol. For microscopic examination, the experimental epidermal peels were stained with Safranin O for about five minutes and mounted on clean microscopic glass slide in 25% glycerol (Saheed and Illoh, 2010; Saheed *et al.*, 2017).

Data analysis

Data obtained from the study were subjected to Analysis of Variance using PAST software. Means were separated using Least significance difference (LSD) with the level of testing significance set at 0.05%.

Results and Discussion

Figure 1 and Tables 1 and 2 illustrate the effects of simulated acid rain on morphology and stomatal traits of *C. argentea* and *C. olitorius*, respectively.

In *C. argentea*, a general reduction in morphological characters was observed in the plants exposed to simulated acid rain when compared with the control counterparts. Leaf numbers were significantly ($p < 0.05$) reduced from 16.5±1.1 in treated plants to 7.7±0.6 in control and leaf area equally reduces significantly ($p < 0.05$) from 18.7±1.1 to 9.0±0.8 in treated to control respectively. However, marginal increase in petiole length as well as the decrease in plant height were not significantly ($p < 0.05$) different between the treated and control (Table 1). Interestingly, changes in all the morphological attributes of *C. olitorius* investigated were found not to be significantly different ($p < 0.05$) between the treated plants and the control (Table 2).

Table 1: Effect of simulated acid rain (SAR) on stomatal traits and morphological characters of *Celosia argentea* L.*

Treatment	Stomatal index (%)		Guard cell area (μm2)		Plant height (cm)	Leaf number	Leaf area (cm^2)	Petiole length (cm)
	AD**	AB	AD	AB				
Control	18.1±1.0b***	25.1±1.1a	100.1±2.5a	76.1±1.8a	14.93±1.7a	16.5±1.1a	18.7±1.1a	2.0±0.1a
SAR-Treated	26.7±1.3a	10.4±1.2b	67.4±0.8b	64.6±1.0b	13.99±1.7a	7.7±0.6b	9.0±0.8b	2.3±0.1a

*** Values are means of ten replicates (n=10); ** AD= Adaxial; AB= Abaxial; *** Similar letters on values along the column are not significantly different at p<0.05**

Table 2: Effect of simulated acid rain (SAR) on stomatal traits and morphological characters of *Corchorus olitorius* L.[*]

Treatment	Stomatal Index (%)		Guard cell area (μm^2)		Plant height (cm)	Leaf number	Leaf area (cm^2)	Petiole length (cm)
	AD[**]	AB	AD	AB				
Control	23.2±4.3a[***]	9.3±4.8a	84.2±27.2a	98.5±13.6a	27.3±1.7 a	9.5±0.6 a	12.9±1.0a	2.6±0.2 a
SAR-Treated	8.0 ±1.8b	5.1±2.6b	22.4±6.6b	22.4±6.6b	26.7±1.8 a	10.1±0.7 a	15.4±1.1a	2.9 ±0.2 a

[*] Values are means of ten replicates (n=10); [**] AD= Adaxial; AB= Abaxial; [***] Similar letters on values along the column are not significantly different at p<0.05

Examination of the leaf epidermal characters generally revealed clear attributes of the surface morphology and distinct stomatal traits. Epidermal cells on the adaxial surface of both species are generally irregularly shaped with slight to deeply wavy or sinuous anticlinal walls (Figs 1A, C, E and G). However those on adaxial surface of the two species are mostly polygonal rarely irregular in shape, with anticlinal walls that are generally straight, few are slightly wavy (Figs. 1B, D, F and H). Stomata types are anomocytic to anisocytic on both surfaces of the two species investigated. The variation in the preponderance of stomata and the stomatal pore (guard cell area) between the control and the treated plant surfaces could be easily determined here (Fig. 1). Crystals in the form of druses of calcium oxalate and crystal sands are present and randomly distributed more in treated plant surfaces than those of the control.

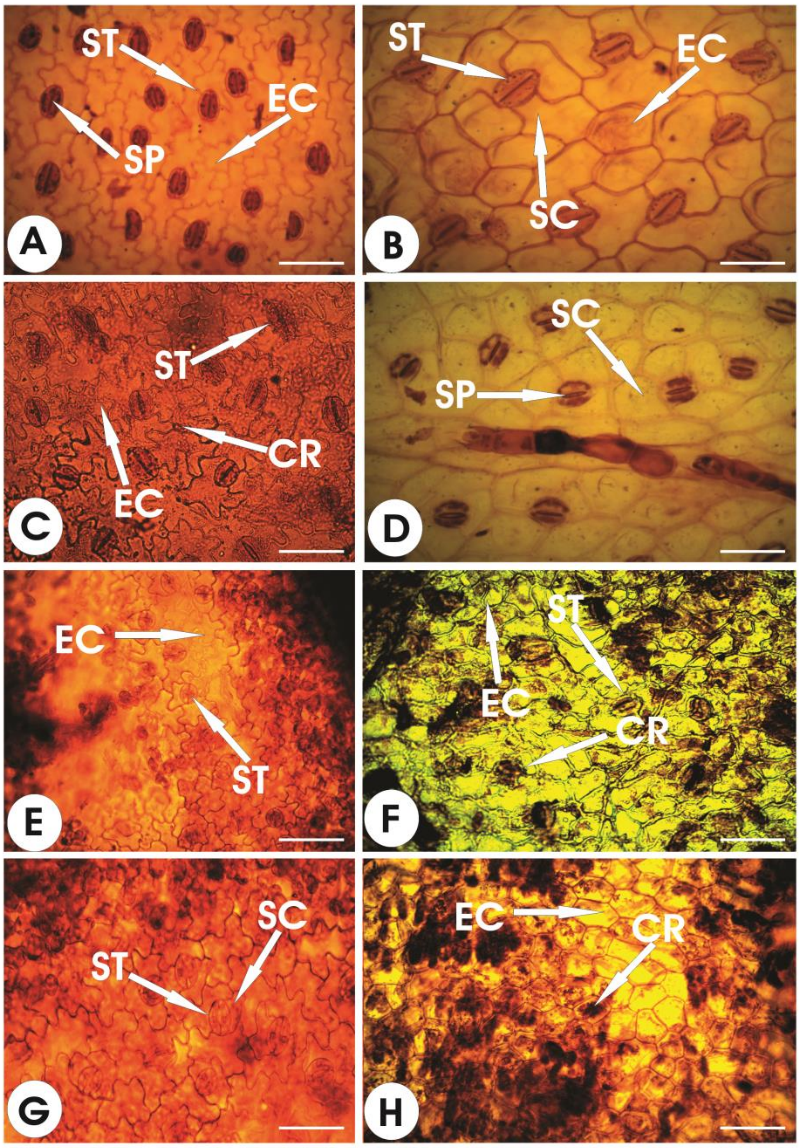

Fig. 1: Adaxial and abaxial epidermal surfaces of control and simulated acid rain treated *Celosia argentea* and *Corchorus olitorius*
A–D is *C. argentea*: A = Abaxial Control; B = Adaxial Control; C = Abaxial Treated; D = Adaxial Treated; E–H is *C. olitorius*: E = Abaxial Control; F = Adaxial Control; G = Abaxial Treated; H = Adaxial Treated. ST – stomata, SP – Stomatal pore, EC – epidermal cell, SC – subsidiary cell, CR – crystals sand, D- druses. Scale =30 μm

Quantitatively, significant reductions ($p < 0.05$) were observed in all the stomata traits (stomatal index and guard cell area) of adaxial and abaxial surfaces in *C. argentea* and *C. olitorius*, of the treated when compared to the control plants (Tables 1 and 2). For example, the stomata index reduced from 25.1±1.1% in abaxial surface of the control plants of *C. argentea* to 10.4±1.2% in the treated plants (Table 1), while the reduction was from 9.3±4.8 to 5.1±2.6% on the same surface of control and treated in *C. olitorius* (Table 2), respectively. The same pattern of reduction was observed for the adaxial surface of *C. olitorius* (Table 2). However, an exception to this observation was recorded on the adaxial surface of *C. argentea* (Table 1), where there is an observed increase in the stomatal index in the treated (26.7±1.3%) when compared to the control (18.1±1.0%). The same trend was observed in the guard cell area on both surfaces of the two investigated species. A significant reduction ($p < 0.05$) of the guard cell area was recorded on both abaxial and adaxial surfaces of C. *argentea*, where the area reduces from 100.1±2.5 to 67.4±0.8 μm^2 in control and treated adaxial surface respectively (Table 1) while the similar surface of *C. olitorius* showed a huge reduction from 84.2±27.2 to 22.4±6.6 μm^2 in control and treated plants, respectively (Table 2).

Several studies have investigated the extent of injuries caused by the exposure of plants to pollutants most especially acid rain (Gabara *et al.*, 2003; Silva *et al.*, 2005; Sant'Anna-Santos *et al.*, 2006). The severity of these injuries often depends on the age of the plants, internal structures, stomatal traits, leaf indumentum as well as environmental factors (Dickison, 2000). In this study, we investigated the effect of SAR on leaf morphology and stomatal traits of *C. argentea* and *C. olitorius*. In *C. argentea*, the SAR condition impacted negatively on the leaf morphology presented as reduction in plant height, number of leaves and leaf area. Observations from the current study agree with several reports on the effect of SAR on plants such as *Clausena lansium, Pisum sativum, Zinnia elegans,* and *Lagerstroemia speciosa* (Kloseiko *et al.*, 2001; Xiao *et al.*, 2004; Tyagi *et al.*, 2004; Dhaka, 2006). Increase in petiole length observed might be due to low exposure of the structure to SAR. On the contrary, marginal increase was observed in all the morphological characters investigated in *C. olitorius.* This implies that the application of SAR did not elicit any deleterious effect on this plant. This observation is evidence that exposure to SAR can sometimes confer advantage to plants when it comes to biomass production. A similar result was observed in *Vicia faba* where a combination of sulfur dioxide and acid rain did not show a deleterious effect on the leaf morphology (Adaros *et al.*, 1988). Another important factor might be due to the nature of the leaves of *C. olitorius* that are extremely fibrous which prevented acidic component of SAR from penetrating the leaves. The leaves are also known for their ability to produce stress tolerance agents like calcium oxalate-crystal and phytoalexins, thereby allowing *C. olitoriu*s plants ability to withstand SAR exposure (Zeid, 2002; Mazen, 2004).

Stomata are the principal means of gas exchange in vascular plants. They are small pores found epistomatically, hypostomatically, and amphistomatically on leaves that are fully or partly opened or closed under the control of a pair of kidney-shaped guard cells (Fitter and Hay, 1978; Grant and Valnick, 1998; Adedeji and Jewoola, 2008). Regarding the effect of SAR on stomatal traits, we observed a significant reduction in stomatal index and guard cell area in the two plants on the two surfaces in the treated group when compared with the control. The only exception is the stomatal index of the adaxial surface of *C. argentea* which increased in the SAR-treated plants (Table 1). This result is contradictory to the previous studies on the effect of pollution on stomatal traits, where an increase in stomatal density and guard cell area was observed when the leaves are exposed to pollutants (Kapitonova, 2002; Gostin, 2009; Ogunkunle *et al.*, 2013). Interestingly, Saheed *et al.* (2017) also observed a decrease in stomatal index in *Talinum triangulare* when exposed to polluted soils from a smelting factory dumpsite. The increase in stomatal index on the adaxial surfaces provides clear evidence that the imposed SAR condition caused a lot of damage to leaves of *C. argentea* compared to those of *C. olitorius.* This is because *C. argentea* had to allocate more resources to stomatal production at the adaxial surfaces that are more exposed to SAR, thus allowing leaves to tap ample CO_2 needed for sugar production in the photosynthetic tissues.

Conclusion

This study elucidated on how *Celosia argentea* and *Corchorus olitorius* responded individually to applied SAR conditions. There are clear pointers in this report that the two plants differ in their responses to SAR. The exposure is advantageous to *C. olitorius* as its growth and leaf morphological characters were not apparently inhibited during the experiment. However, *C. argentea* appears to be susceptible to SAR. The possible explanation for the response of *C. olitorius* to SAR is the reduction in stomatal index and guard cell area at the adaxial surfaces of the leaves, which may be adaptations for reduced transpiration to prevent water and energy loss. Another reason might be the ability of *Corchorus* to produce stress tolerance agents like calcium oxalate crystals and phytoalexins on its leaf surfaces.

Conflict of Interest

The authors declare that there is no conflict of interest reported in this work.

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