Title- Ecology of Leaf Adaptations in *Azardirachta indica* A. Juss at Desert Environment

Journal- Research & Review: Journal of Ecology.

ISSN- 2278-2230, Volume- 13, Issue 1, Year 2024.

Article Received- March 29, 2024

Article Approved- April 19, 2024

Article Published-

Article Type- Research

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ABSTRACT

Azardirachta indica A. Juss is a medicinally important plant belonging to the family Meliaceae. It is widely used during COVID 19 for its anti-inflammatory and antiviral effects for COVID 19 prophylaxis. Although the plant is native to India, but is very much naturalized in tropical and subtropical countries. It is one of the commonly used plant for shelterbelt and wind break system along roadsides also. The present investigation was done to check the adaptive strategies followed by this plant to survive in arid and semiarid region of Rajasthan. Micromorphological characters viz., Stomata type, Stomata Index, Stomatal density, Stomata length and width, Epidermal cell shape and wall pattern with epidermal cell length and width was estimated by leaf Impression method. Micrometry was done using Magnus Pro software fitted on a light microscope of Cannon, Ecophysiological parameters by Li Cor photosynthetic portable system and succulence by method proposed by A. Mantovani (1998), alternative to Delf's index. Micromorphological variations in young and mature leaves were showed in tabular form for both the leaf surfaces. Mature leaves were observed to be more succulent in comparison to young leaves and there were no marked variations in water use efficiency in the leaves of both young and mature leaves which make it more hospitable to survive in xeric conditions. The research conducted on Azadirachta indica A. Juss explores its adaptation mechanisms in arid and semiarid regions of

Rajasthan. Through micromorphological analysis and ecophysiological measurements, the study reveals the plant's resilience strategies, particularly in terms of leaf characteristics and water use efficiency. These findings contribute to understanding its suitability for diverse environmental conditions, including its potential significance amidst the COVID-19 pandemic for its medicinal properties.

KEYWORDS- Water Use Efficiency, Micromorphological characters, Succulence, Adaxial surface and Abaxial surface.

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INTRODUCTION

Azardirachta indica A. Juss is a multipurpose tree which has been found as core species for desert afforestation in India because it is adapted to tropical and subtropical climates and can tolerate high summer temperatures even up to 50 °C. It's cultivated in windbreaks to shield food crops from desert winds, utilized in shelterbelt forests to diminish wind speed, and aids in the ecological renewal of depleted mining sites. It has been found as core species for afforestation in Indian desert and is used for supplying food, fodder, fuel, timber, medicine and for providing shade and shelter to desert inhabitants [6]

Neem trees has been commercially grown for the production of azadirachtin, which is a ecofriendly insecticide, because synthetic pesticides adversely affect environment, health and biodiversity [2]

Neem has been found as considerable interest, as neem has been generated as a means to prevent the spread of deserts and improve desert environments in Saudi Arabia [1], sub-Saharan Africa [13] and western India [4]. In view of the role of neem in water harvesting and conservation research on establishment in Western India where low and erratic rainfall, high potential evapotranspiration and high wind speeds prevail [4,5].

Aiming to expand the knowledge on this, this work has dealt to analyse the micromorphological characters, succulence and water use efficiency to check the adaptive strategies followed by *Azardirachta indica*.

MATERIALS AND METHODS

The experiment was conducted at the Botanical Garden of Jai Narain Vyas University, Jodhpur (North Lattitude 26° 0´ & 27° 37´, East Longitude 72° 55´ & 73°52´) during the full sunny day.

Succulence estimation

Ten leaves each from young (2nd node) and mature (6th node) plants, sourced from various individuals, were gathered, promptly sealed in plastic bags, and transported to the laboratory. In the laboratory, the leaves were cut at the base of their petioles, and the fresh weights (FW) of the leaves were measured. Then leaves were placed inside moistened plastic bags, for 12 h at 4°C to allow water imbibitions. Afterwards, each leaf was reweighed and the value recorded as maximum fresh weigh (MFW). Leaf shape was drawn on graph paper to calculate the leaf area (A). Finally, leaves were oven-dried for two days at 60°C, a period pre-determined as sufficient to obtain its constant dry weight (DW).

Leaf succulence was estimated using method proposed by Mantovani [8] alternative to Delf's index.

$$LS = MFW-DW/A$$

Estimation of Micromorphological characters:

Ten –ten leaves each of young (2^{nd} node) and mature (6^{th} node) were taken for investigation to study micromorphological characters of abaxial and adaxial surfaces [Fig. 1 and 2].

Slide preparation for micromorphological studies

Impression of epidermal peels from the leaves were obtained by leaf impression method [10]. Leaves impressions were washed with water, stained in safranin, and mounted on 50% glycerine. Fifteen observations of each peel covering the entire leaves were recorded for all the species and each slide was observed for 15 microscopic fields for both the surfaces.

Determination of stomata complex type and stomata density

Using 35 fields of view at X 40 objective as quadrats, the number of subsidiary cells per stoma was noted to determine the types of stomata complex present in each specimen. Terminologies given by [12] and [16] were used for stomatal complex types. The stomatal density (SD) was determined as the number of stomata per square millimeter [16].

Determination of Stomata Index and Stomata size (Length and Width)

Stomatal index (SI) was determined as follows:

$$SI = (S/E+S) \times 100$$

Where: SI = Stomatal index

S = Number of stomatal per square millimetre

E = Number of ordinary epidermal cells per square mill imeter.

The mean stomata size i.e. Stomata length and width was determined by using Magnus pro software fitted on to a light microscope of Olympus CH20i- TR

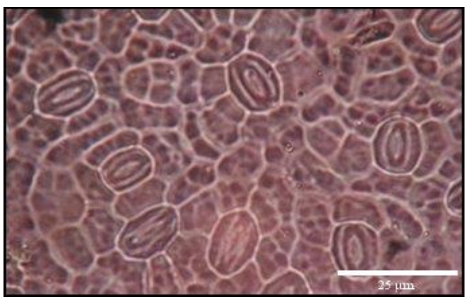

Fig. 1 Abaxial surface

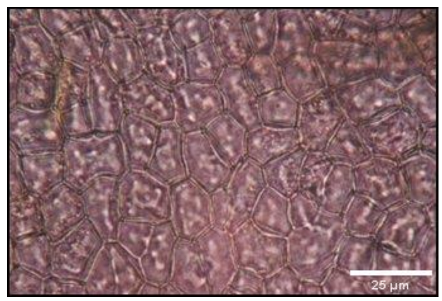

Fig. 2 Adaxial surface

Determination of Transpiration rate

Transpiration rate was determined by using the portable photosynthesis system LI-6400 (LI-COR Inc., Lincoln, USA; upgraded with internal CO_2 source for controlled calibrations and light source adjustments). Transpiration rate was expressed as $mol/m^{-2}/s^{-1}$.

Statistical Analysis

Data from the microscopic studies and transpiration rate were subjected to statistical studies of Mean and Standard Error using Microsoft excel 2007.

RESULTS:

Leaves of *Azardirachta indica* are amphistomatic, that is, stomata are present on both adaxial and abaxial surfaces of young and mature leaves, but number of stomata was more on abaxial leaves surfaces in both types. They have anomocytic type of stomata at abaxial and adaxial side (Table 1).

- Length and width of stomata is more at abaxial side in both young and mature leaves.
- The highest stomatal index was found on the underside of both young and mature leaves.
- The epidermal cells were pentagonal in shape with straight arched wall in both young and mature leaves. Length of epidermal cell is more on abaxial side in young leaves and in mature leaves it is more on adaxial side, width of epidermal cells in young leaves is more on adaxial side whereas in mature leaves it is more on abaxial side.
- Trichomes are absent in *Azardirachta indica.*
- Water use efficiency does not show marked variations in young and mature leaves.
- Mature leaves are 4652% more succulent as compared to young leaves

DISCUSSION

In amphistomatous leaves, stomatal frequency is usually greater in the lower leaf surface than in the adaxial side [18,17] and in arid environment amphistomatous leaves are most common. *Azardirachta indica* is the most frequently used plant in shelterbelt and windbreak system in arid region.

Stomata are the turgor operated valve for exchange of gases and water vapours, they manage their opening and closing with respect to the external environmental conditions. Changes in stomatal density, stomatal size and stomatal location on the leaf surface in response to varying environmental conditions can affect stomatal conductance [7], and thereby carbon assimilation, because there is a close relationship between photosynthesis and stomatal conductance [9]. The stomatal size has positive correlation with transpiration rate and *Azardirachta indica* represented small stomata size and stomatal index and hence small transpiration rate [15] and thereby more WUE.

Succulent leaves possess abundant water content, along with resilient cell walls and apoplastic polysaccharides, enabling them to maintain high leaf water potentials even under significant dehydration[11,14].

This function acts as a safeguard against excessive water loss through transpiration, extending the duration of stomatal opening and facilitating beneficial gas exchange. The extent of this effect corresponds to the level of leaf succulence. [3,11] This is likely why there was no significant difference observed in the Water Use Efficiency (WUE) between young and mature leaves in this study. Mature leaves are thousands times more succulent than young leaves and manages the water status in plant.

Table 1. Micromorphological, water use efficiency and succulence of young and mature leaves of *Azardirachta indica*

Parameters	Young Leaves		Mature Leaves	
	Abaxial surface (Mean ± SE)	Adaxial surface (Mean ± SE)	Abaxial surface (Mean ± SE)	Adaxial surface (Mean ± SE)
Stomatal Type	Anomocytic	Anomocytic	Anomocytic	Anomocytic
Stomatal Index (%)	17.4324 ± 0.2717	0.3333 ± 0.3333	18.6447 ± 0.1880	3.3433 ± 0.1595
Stomatal density (per mm^2)	13.6667 ± 0.3333	0.3333 ± 0.3333	11.0000 ± 0.5774	1.6667 ± 0.3333
Stomata Length (µ)	12.5317 ± 0.0086	5.1711 ± 2.5868	14.8748 ± 0.0119	7.9620 ± 0.0093
Stomata width (µ)	8.2335 ± 0.0070	3.4908 ± 1.7455	9.3016 ± 0.0073	2.8706 ± 0.0085
Epidermal Cell Shape & wall Pattern	Pentagonal with straight arched wall	Pentagonal with straight arched wall	Pentagonal with straight arched wall	Pentagonal with straight arched wall
Epidermal Cell Length (µ)	9.6638 ± 0.0096	7.6611 ± 3.8307	11.3013 ± 0.0095	14.1586 ± 0.0125
Epidermal Cell Width (µ)	5.4264 ± 0.0055	6.2806 ± 3.1404	9.0966 ± 0.0107	8.5541 ± 0.0225
Trichome Type	Absent		Absent	
Water Use Efficiency	2.0918		2.5878	
Succulence	0.004		0.174	

CONCLUSION

It is the highly adapted plant for the harsh climatic conditions of arid and semi-arid region and hence extensively used for shelterbelt and agroforestry in Western Rajasthan.

ACKNOWLEDGEMENT

The authors acknowledge the Department of Botany, UGC-CAS and DST-FIST for facilities.

CONFLICT OF INTEREST

The author declares no conflict of interest whatsoever.

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