Research & Reviews in BioSciences

Research | Vol 11 Iss 3

Foliar Epidermal Study of *Withania somnifera* Grown in Heavy Metal Treated Soil

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Received: September 16, 2016; **Accepted:** November 2, 2016; **Published:** November 12, 2016

Abstract

***Withania somnifera* L. Dunal is one of the most important medicinal plants and also known as "Indian ginseng". The present study deals with pot culture experiments with plants were grown in three treatments in black soil, Treatment No I. control without any addition to the soil, Treatment No II. Cadmium 10 ppm, Chromium 20 ppm, Nickel 16 ppm were introduced into the soil, Treatment No III one % of Calcium hydroxide was also added along with heavy metals to soil. Then plants were grown up to the productivity levels. The micro morphological characters of *Withania somnifera* in epidermal cells were polygonal on the both surfaces, anticlinal walls were straight and smooth surface were dominant on both the surfaces, which are same in three treatments. The costal cells are present on the both surfaces as they are parallelly were dominant on both surfaces in three treatments. The epidermal cells frequency differs a lot within the same surface and also on both the surfaces of the same leaf of *Withania somnifera*. Highest epidermal frequency is dominated at middle zone on abaxial surfaces in three treatments. It was observed anisocytic stomata are dominant on both the surface, which are same in three treatments. The (Treatment I) control plants showed high stomatal index on abaxial surface in leaf apex, lamina and margin compared to the (Treatment II) heavy metal treated plants and (Treatment III) heavy metal +1% $Ca(OH)_2$ treated plants. Trichome density was supported maximum on abaxial surface in three treatments. The present investigation highlights about shape, anticlinal walls, thickness of walls, surface characters and distribution of epidermal cells, epidermal frequency, type of stomata, stomatal frequency, stomata index, type of subsidiaries arrangement and orientation of stomata and trichomes density in different three treatments.**

Keywords: ***Withania somnifera; Foliar epidermis; Micro morphology; Stomata; Costal cells; Trichome complex***

Introduction

Withania somnifera L. Dunal is an important medicinal plant belongs to the division Magnoliophyta, class magnoliopsida, order solanales and family Solanaceae [1], commonly known in India as Ashwagandha or winter cherry (vernacular: Sanskrit: Ashwagandha; Telugu: Panneru; Trade name: Ashwagandha) is used in more than 100 formulations of Ayurveda, Unani and

Citation: Saidulu CH, Venkateshwar C, Gangadhar Rao S, et al. Foliar epidermal study of Withania somnifera grown in heavy metal treated soil. Res Rev Biosci. 2016;11(3):107.

Siddha and is therapeutically equivalent to ginseng [2]. It is known classically for its rejuvenating properties, and hence called "Indian Ginseng" [3]. It has been used as an antibacterial, antioxidant, adaptogen, aphrodisiac, liver tonic, anti-inflammatory agent [4]. Numerous studies indicated that ashwagandha possesses antitumor, antistress, immunomodulatory, hematopoietic, anti-ageing, anxiolytic, ant-depressive rejuvenating properties and also influences various neurotransmitter receptors in the central nervous system [5]. *Withania somnifera* is anxiety, insomnia, respiratory disorders including emphysema, asthma bronchitis and coughs [6]. It is an ingredient in many formulations prescribed for a variety of musculoskeletal conditions (e.g., arthritis, rheumatism), and as a general tonic to increase energy, improve overall health and longevity, and prevent disease in athletes, the elderly, and during pregnancy. The anatomical studies of *W. somnifera*, the leaves are dorsiventral with prominent midrib and bilaterally symmetrical lamina [7-9]. Dense glandular and eglandular hairs on the surfaces of both epidermises [10].

Importance of epidermal characters in taxonomy of the living as well as fossil angiosperms and in pharmacognosy and forensic science is well known [10-16]. Plant leaves by virtue of their position, shape and texture, they get exposed to various environmental conditions, hence, leaf epidermis play a vital role as it interacts with the exiting environment and get acclimatized to the exposed environment with modification within the epidermis [17,18].

Environmental impact assessment studies have become most important in recent days therefore effects of pollutants on leaves can be studies well, because leaf is the ultimate source of crop production. Being protective in nature, the epidermis gets exposed first to the pollutants and it responds either in positive or in negative i.e., it explains the tolerance or intolerance. In aspects, the leaves/barks/wood are the best accumulators of pollutants in them, thereby the toxic metal accumulation leads to the defective architecture of the leaf epidermis and depositions in them.

The information regarding the various aspects of epidermal cell complex and costal cells, like shape of cells, anticlinal walls, surface characters, orientation, arrangement and distribution is negligible. Hence, to provide concrete data on medicinal plant epidermal cell complexes of leaf has been evaluated by micro-morphological study of leaf epidermal cell complex. The objectives of the present study deals with the heavy metal toxicity assessed the epidermal cell complex for which is too essential for the evaluation of the toxicity. The epidermal cells are important tool for identification, adulteration and authentication of crude leaf drugs of *Withania somnifera*.

Material and Methods

Plant material source

Withania somnifera seeds were procured from the CIMAP, Hyderabad. The seeds were sown in earthen pots at green house of botanical garden, Department of Botany, Osmania University, Hyderabad and India.

Experimental material and design of experiment

Layer of soil (0 cm to 15 cm) was taken from clean area of Botanical Garden, Department of Botany, Osmania University, Hyderabad, Telangana State and India. The soil was a sandy loam semi black earth, and freshly collected soil was passed through a 2 mm sieve and air dried for one week, then experimental soil was taken and filled into 15 pots for individual exposure. Treatment No I control without any addition to the soil. Treatment No II Cadmium 10 ppm, Chromium 20 ppm, Nickel 16 ppm were introduced into the soil. Treatment No III one % of Calcium hydroxide was also added along with heavy

metals to soil. Plants were grown in pot culture experiments with three treatments and were grown up to the productivity levels during.

Surface study

The mature 10 plants (leaves/plant) were selected randomly were collected from three treatments in plants of mature leaves and fixed in carnou's fixative consisting of alcohol and glacial acetic acid 3:1 [19], after two days, the fixative was replaced by 70% alcohol for preserving the material FIG. 1.

Carefully both the upper and lower epidermis were peeled off by fine hand sections of leaf epidermis were stained with Safranin and mounted in glycerin. The microphotographs were taken by using microscopic LEICA LS2 binocular research microscope with Magnus camera 2 mega pixels attached to the microscope. A minimum of 10 readings were taken in each case to calculated the average frequencies of stomata, epidermal cells, stomatal index, stomata types and trichomes. The standard techniques were employed, the scraping method [20] and "Triple acid method [5] which gave satisfactory results for the preparation of epidermal peelings. The peels were prepared from base, apex, midrib, lamina and margin locations of the leaf, for both the surfaces [13].

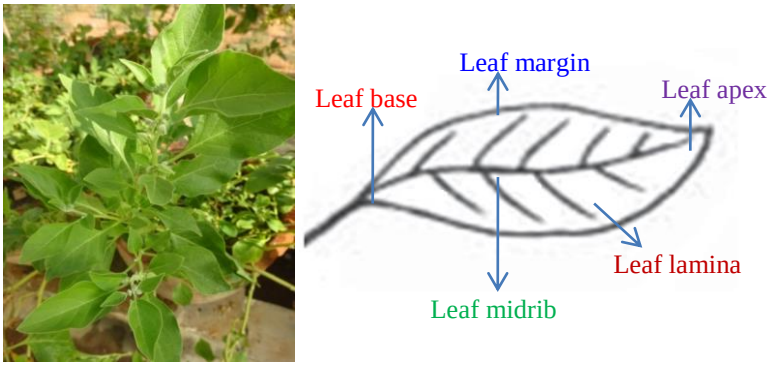


FIG. 1. ***Withania somnifera*** **plant and leaf.**

Stomatal index

Number of stomata and stomatal index of the both surfaces of leaves were scientifically observed and values taken by trial and error method as per API standard [21]. Stomatal frequency, Stomatal index values are averages derived from ten readings. Stomatal index's (S.I) were calculated following Salisbury formula (1927) as given below:

$$\text{Stomatal Index} = \frac{\text{No of stomata}}{\text{No. of Stomata} + \text{No. of Epidermal cells}} \times 100$$

Result and Discussion

Epidermal cell complex

Epidermal cells: In the epidermal cells the three treatments studies have not shown much significant difference. The shapes of the epidermal cells are described to be polygonal anisodiametric or nonlinear and anticlinal walls sides mostly straight, outer walls flat, surface is smooth, cytoplasmic contents are dense, distribution irregularly arranged, variously oriented on both surface adaxial and abaxial of *Withania somnifera.*

Epidermal frequency: The frequency of epidermal cells differs on both the surfaces and also within the same surface and at various locations of the same leaf i.e., at leaf base, leaf midrib, leaf apex, leaf lamina and leaf margin.

In *Withania somnifera* leaf, the epidermal cell frequency at leaf base was 56358.38 per cm^2 (adaxial) and 5794.97 per cm^2 (abaxial), at leaf apex was 55346.82 per cm^2 (adaxial) and 49132.94 per cm^2 (abaxial), at leaf midrib was 59537.57 per cm^2 (adaxial) and 55057.80 per cm^2 (abaxial), at leaf lamina was 60115.60 per cm^2 (adaxial) and 57803.46 per cm^2 (abaxial), and at leaf margin was 58959.53 per cm^2, (adaxial) and 61271.6 per cm^2 (abaxial) in plants were grown in control soils. And plants grown in heavy metal treated soils it was observed that the leaf epidermal cell frequency at leaf base was 57803.46 per cm^2 (adaxial) and 62138.72 per cm^2 (abaxial), at leaf apex was 57369.94 per cm^2 (adaxial) and 59248.55 per cm^2 (abaxial), at leaf midrib was 64739.88 per cm^2 (adaxial) and 70375.72 per cm^2 (abaxial), at leaf lamina was 61994.21 per cm^2 (adaxial) and 59971.09 per cm^2 (abaxial), and at leaf margin was 53757.22 per cm^2 (adaxial) and 55635.83 per cm^2 (abaxial). In plants grown with heavy metal +1% Ca $(OH)_2$ treated soils leaf epidermal cell frequency at base was 55635.83 per cm^2 (adaxial) and 56213.87 per cm^2 (abaxial), at leaf apex was 50289.01 per cm^2, (adaxial) and 47832.36 per cm^2 (abaxial), at leaf midrib was 57080.92 per cm^2 (adaxial) and 56647.39 per cm^2 (abaxial), at leaf lamina was 54335.26 per cm^2 (adaxial) and 57947.97 per cm^2 (abaxial), and at leaf margin was 56213.87 per cm^2 (adaxial) and 58959.53 per cm^2 (abaxial).

Stomatal complex

Stomatal cells: In the stomata cells the three treatments studies have not shown much significant difference. It was observed that anisocytic stomata is dominant and anticlinal walls sides mostly straight, outer walls concave, surface is smooth, cytoplasmic contents are dense, distribution irregularly arranged, variously oriented on both surface adaxial and abaxial of *Withania somnifera*.

Stomatal frequency and stomatal index: The frequency of stomatal cells and stomatal index differ on both the surfaces and also within the same surface and at various locations of the same leaf i.e., at leaf base, leaf midrib, leaf apex, leaf lamina and leaf margin in three different treatments of *Withania somnifera* (FIG. 2).

In *Withania somnifera* leaf the stomatal frequency at leaf base was12716.76 per cm^2 (adaxial) and 14595.37 per cm^2 (abaxial), at leaf apex was 15895.95 per cm^2 (adaxial) and 11994.21 per cm^2 (abaxial), at leaf midrib was 13583.81 per cm^2 (adaxial) and 12283.23 per cm^2 (abaxial), at leaf lamina was 15028.90 per cm^2 (adaxial) and 16473.98 per cm^2 (abaxial), at leaf margin was 15028.90 per cm^2 (adaxial) and 16184.97 per cm^2 (abaxial) in plants were grown in control soils. And plants grown in heavy metal treated soils it was observed that the leaf stomatal frequency at leaf base was 10549.13 per cm^2 (adaxial) and 13439.30 per cm^2 (abaxial), at leaf apex was 14739.88 per cm^2 (adaxial) and 11560.69 per cm^2 (abaxial), at leaf midrib was 11560.69 per cm^2 (adaxial) and 10404.62 per cm^2 (abaxial), at leaf lamina was 12861.27 per cm^2 (adaxial) and 14595.37 per cm^2 (abaxial), at leaf margin was 10838.15 per cm^2 (adaxial) and 13439.30 per cm^2 (abaxial). Plants grown in heavy metal + 1% $Ca(OH)_2$ treated soils the leaf stomatal frequency at leaf base was 11271.67 per cm^2 (adaxial) and 12716.76 per cm^2 (abaxial), at leaf apex was 13150.28 per cm^2 (adaxial) and 1184971 per cm^2 (abaxial), at leaf midrib was 12572.25 per cm^2 (adaxial) and 11416.18 per cm^2 (abaxial), at leaf lamina was 13872.83 per cm^2 (adaxial) and 14161.84 per cm^2 (abaxial), at leaf margin was 13150.28 per cm^2 (adaxial) and 13583.81 per cm^2 (abaxial) (FIG. 3).

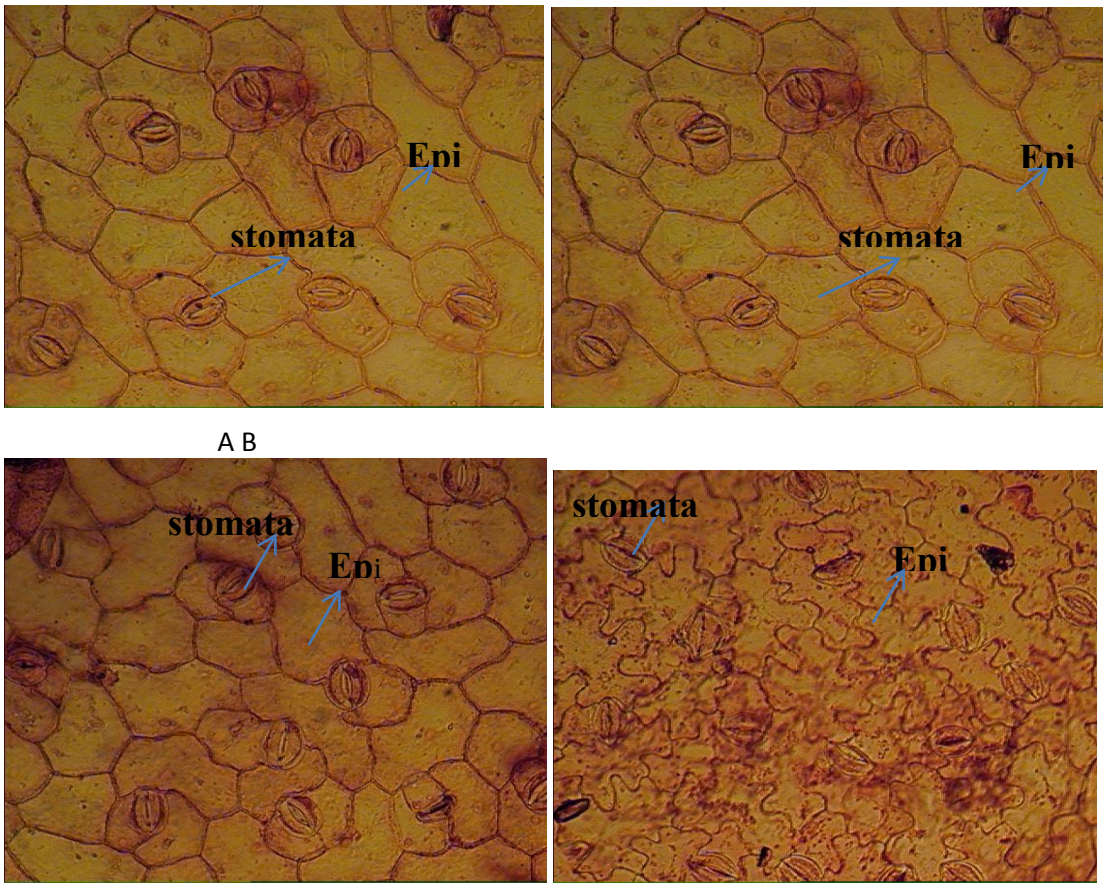
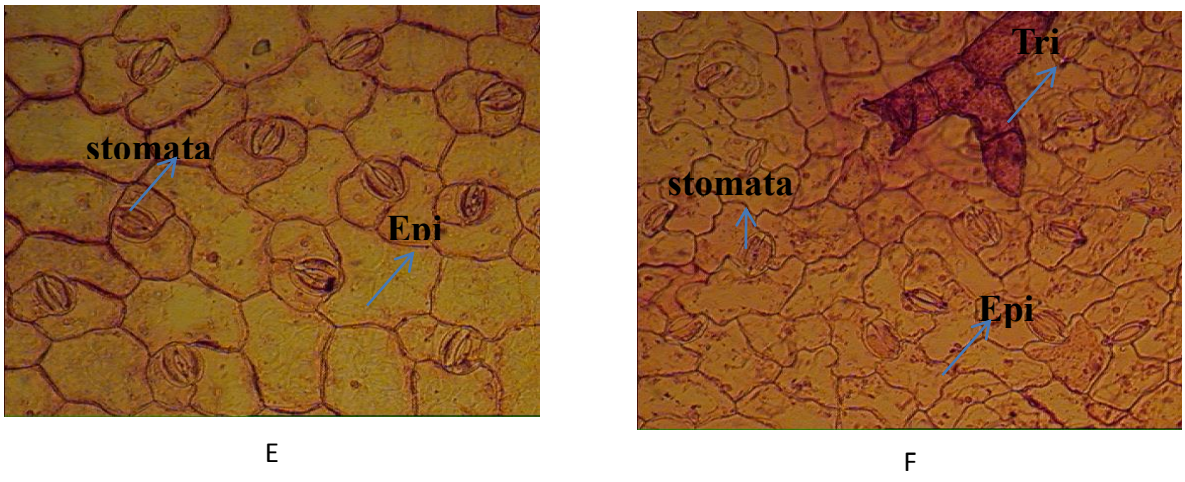


FIG. 2. **Showing the epidermis (adaxial and abaxial surfaces) of *Withania somnifera* leaves in control plants. Leaf apex: A) Ad and B) Ab, Leaf base: C) Ad and D) Ab.**



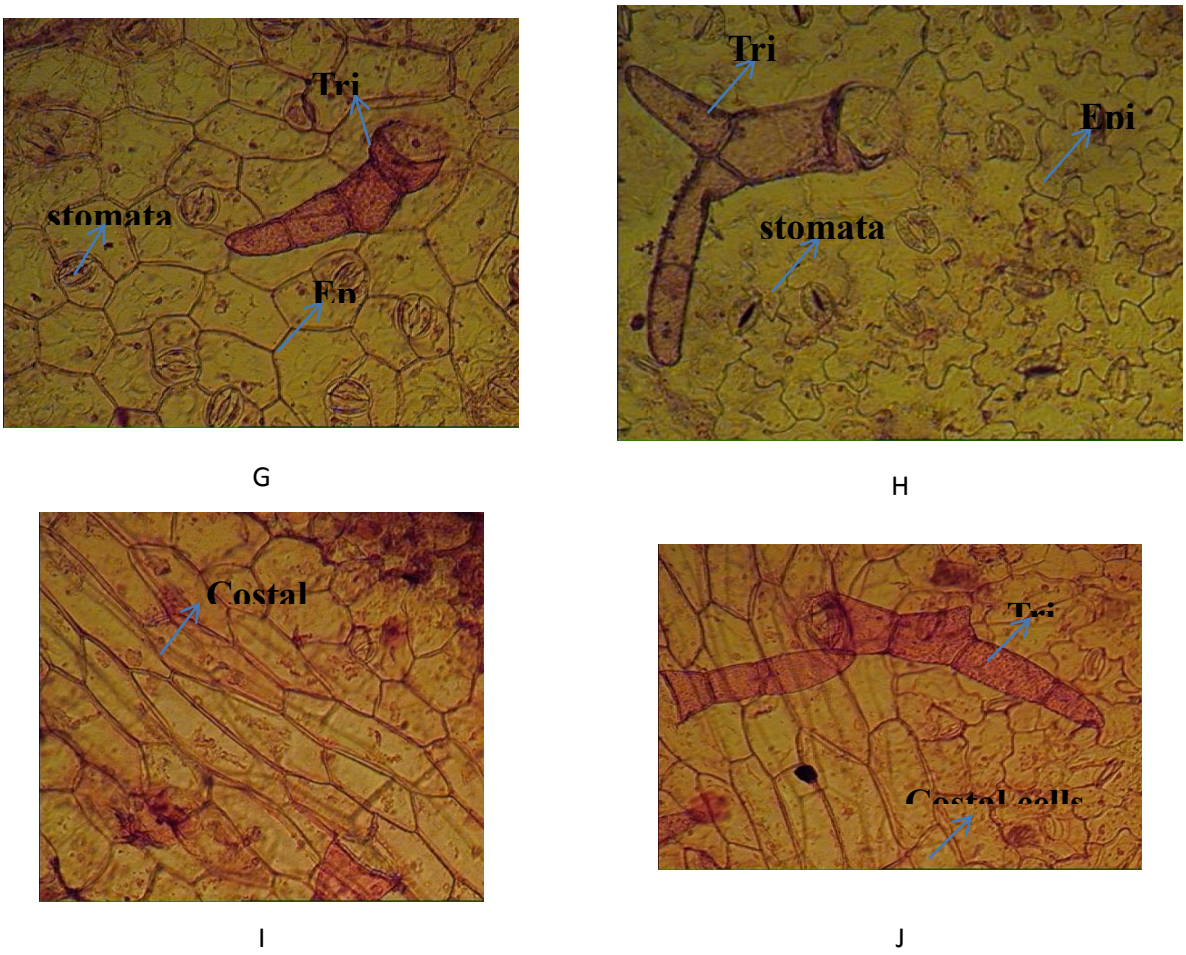


FIG. 3. **The epidermis (adaxial and abaxial surfaces) of *Withania somnifera* leaves in control plants. Leaf lamina: E) Ad and F) Ab, Leaf margin: G) Ad and F) Ab, Leaf midrib: I) Ad and J) Ab.**

In *Withania somnifera* leaf the stomatal index at leaf base was 20.57 cells per unit area (adaxial) and 16.98 cells per unit area (abaxial), at leaf apex was 19.06 cells per unit area (adaxial) and 24.94 cells per unit area (abaxial), at leaf midrib was 16.26 cells per unit area (adaxial) and 17.17 cells per unit area (abaxial), at leaf lamina was 20.33 cells per unit area (adaxial) and 22.17 cells per unit area (abaxial), at leaf margin was 20.31 cells per unit area (adaxial) and 22.53 cells per unit area (abaxial) in plants were grown in control soils. And plants grown in heavy metal treated soils it was observed that the leaf stomatal index at leaf base was 18.86 cells per unit area (adaxial) and 15.40 cells per unit area (abaxial), at leaf apex was17.29 cells per unit area (adaxial) and 19.92 cells per unit area (abaxial), at leaf midrib was 17.34 cells per unit area (adaxial) and 12.88 cells per unit area (abaxial), at leaf lamina was 17.18 cells per unit area (adaxial) and 19.57 cells per unit area (abaxial), at leaf margin was 16.77cells per unit area (adaxial) and 18.56 cells per unit area (abaxial) FIG. 4. In plants grown with heavy metal +1% $Ca(OH)_2$ treated soils the leaf stomatal index at leaf base was 18.60 cells per unit area (adaxial) and 16.70 cells per unit area (abaxial), at leaf apex was 17.27 cells per unit area (adaxial) and 21.11 cells per unit area (abaxial), at leaf midrib was 18.04cells per unit area (adaxial) and 17.81 cells per unit area (abaxial), at leaf lamina was 20.00 cells per unit area (adaxial) and 19.63 cells per unit area (abaxial), at leaf margin was 18.95 (adaxial) and 18.14cells per unit area (abaxial) FIG. 5.

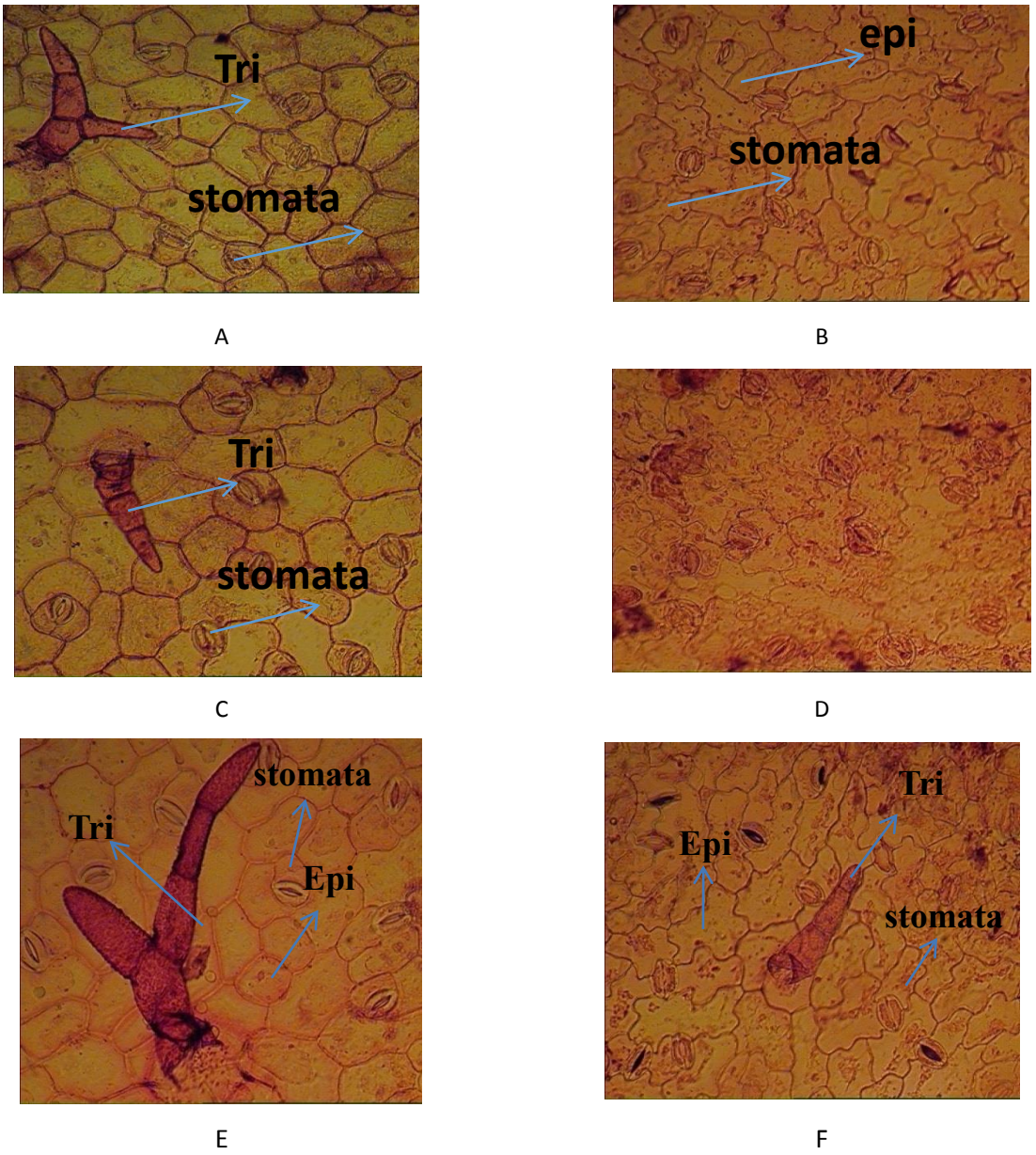
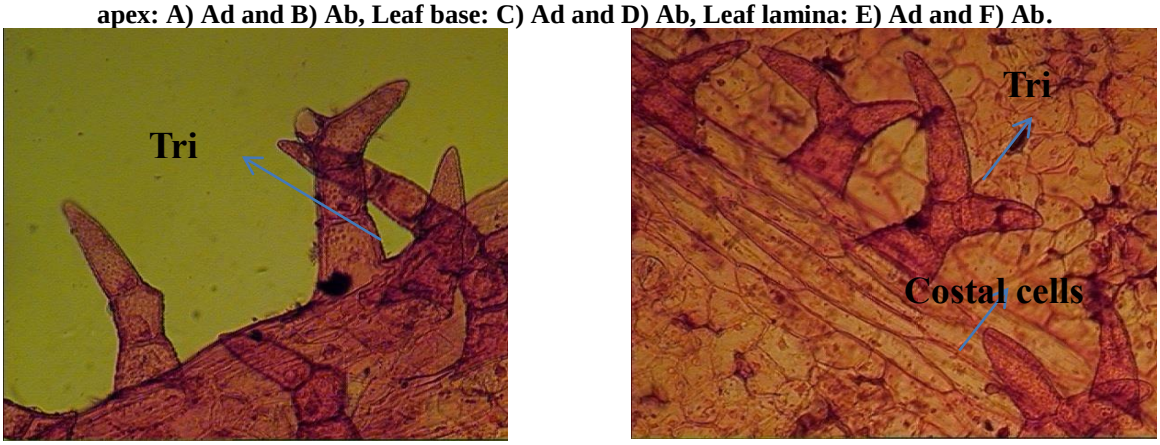


FIG. 4. **the epidermis (adaxial and abaxial surfaces) of *Withania somnifera* leaves in heavy metal treated plants. Leaf apex: A) Ad and B) Ab, Leaf base: C) Ad and D) Ab, Leaf lamina: E) Ad and F) Ab.**



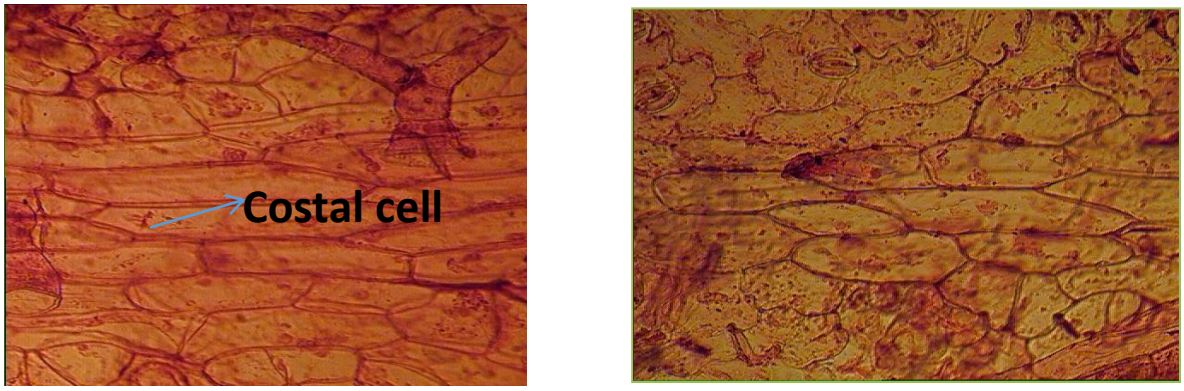


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FIG. 5. **Showing the epidermis (adaxial and abaxial surfaces) of *Withania somnifera* leaves in heavy metal treated plants. Leaf margin: G) Ad and F) Ab, Leaf midrib: I) Ad and J) Ab.**

Note: Epi=Epidermal cells; Tri=Trichomes; Ad=Adaxial surface; Ab=Abaxial surface.

Costal cell complex

In the costal cells the three treatments studies have not shown much significant difference. The shapes of the costal cells are described to be polygonal anisodiametric or nonlinear and anticlinal walls sides mostly straight, outer walls flat, surface is smooth, cytoplasmic contents are dense, distribution irregularly arranged, parallelly oriented on both surface adaxial and abaxial of *Withania somnifera*.

Trichome complex

Trichomes density

The density of trichome cells differ on both the surfaces and also within the same surface and at various locations of the same leaf i.e., at leaf base, leaf midrib, leaf apex, leaf lamina and leaf margin in three different treatments of *Withania somnifera*. In the trichome cells the three treatments studies have not shown much significant difference. The non-glandular trichomes are present in the *Withania somnifera* plants grown in three different treatments.

In *Withania somnifera* leaf the trichomes density at leaf base was 5202.31 per cm^2 (adaxial) and 6069.36 per cm^2 (abaxial), at leaf apex was 4046.24 per cm^2 (adaxial) and 5202.31 per cm^2 (abaxial), at leaf midrib was 3323.69 per cm^2 (adaxial) and 3612.71 per cm^2 (abaxial), at leaf lamina was 4046.24 per cm^2 (adaxial) and 4046.24 per cm^2 (abaxial), at leaf margin was 4624.27 per cm^2 (adaxial) and 4913.29 per cm^2 (abaxial) in plants were grown in control soils. Plants grown in heavy metal treated soils it was observed that the leaf trichomes density at leaf base was 2601.15 per cm^2 (adaxial) and 3612.71 per cm^2 (abaxial), at leaf apex was 3179.19 per cm^2 (adaxial) and 1878.61 per cm^2 (abaxial), at leaf midrib was 2023.12 per cm^2 (adaxial) and 3034.68 per cm^2 (abaxial), at leaf lamina was 3323.69 per cm^2 (adaxial) and 3323.69 per cm^2 (abaxial), at leaf margin was 2601.15 per cm^2 (adaxial) and 4335.26 per cm^2 (abaxial). In plants grown with heavy metal +1% $Ca(OH)_2$ treated soils the leaf trichomes density at leaf base was 3757.22 per cm^2 (adaxial) and 5924.85/cm^2 (abaxial), at leaf apex was 5057.80 per cm^2 (adaxial) and 5346.82 per cm^2 (abaxial), at leaf midrib was 1878.61 per cm^2 (adaxial) and 3179.19 per cm^2 (abaxial), FIG. 6. at leaf lamina was 3612.71 per cm^2 (adaxial) and 3901.73 per cm^2 (abaxial), at leaf margin was 5057.80 per cm^2 (adaxial) and 6936.41 per cm^2 (abaxial) FIG. 7.

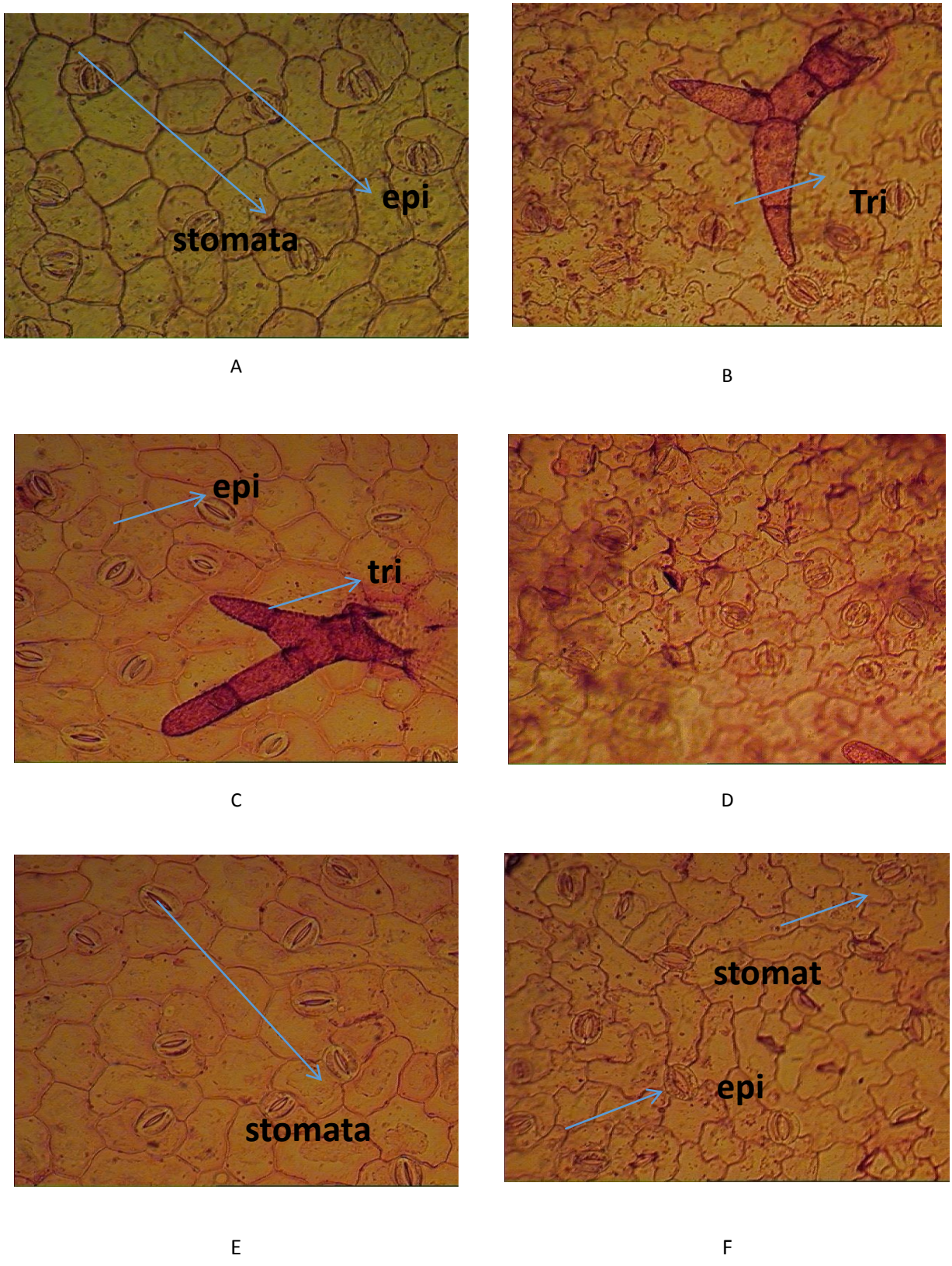

FIG. 6. **Showing the epidermis (adaxial and abaxial surfaces) of *Withania somnifera* leaves in heavy metal +1% $Ca(OH)_2$ treated plants. Leaf apex: A) Ad and B) Ab, Leaf base: C) Ad and D) Ab, Leaf lamina: E) Ad and F) Ab.**

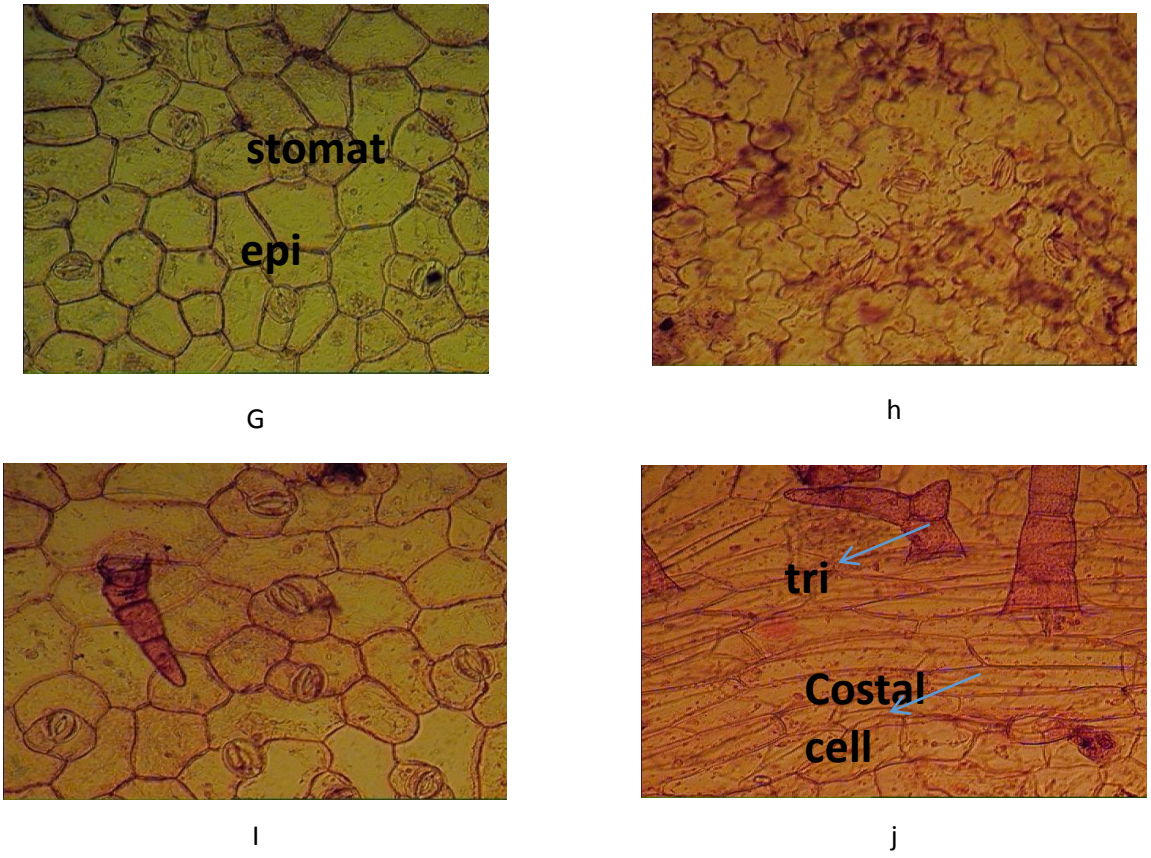

FIG. 7. **Showing the epidermis (adaxial and abaxial surfaces) of *Withania somnifera* leaves in heavy metal +1% $Ca(OH)_2$ treated plants). Leaf margin: G) Ad and F) Ab, Leaf midrib: I) Ad and J) Ab.**

The micro morphological characters of *Withania somnifera* in the epidermal cells were polygonal on the both surfaces, anticlinal walls were straight and smooth surface were dominant on both the surfaces, which are same in three treatments. It was observed anisocytic stomata are dominant on both the surface, which are same in three treatments. Cytoplasmic contents of cells are dense, irregularly arranged, variously oriented on both surface adaxial and abaxial of *Withania somnifera*. In angiosperms in uses of epidermal cells are usually said to be less conspicuous on the leaf lamina adaxial than on abaxial [22,23,14], which is confirmed in the present study. Anticlinal walls of foliar epidermal cells are usually straight in tree members and sinuate in herbs [13,14]. Anticlinal walls in some tree members and straight anticlinal walls in herbaceous member which differed by the study [14].

The epidermal cell frequency was showed maximum in plants grown in heavy metal treated soils (Treatment No. I) leaf base, apex, midrib and lamina on both surface and also minimum at leaf margin on both surface as compared to other treatments. The epidermal frequency is observed maximum on abaxial surface of leaf midrib in plants grown in heavy metal treated soils (Treatment No. I), while it is minimum at leaf apex of abaxial surface of control plants. Foliar epidermis Solanaceae family has been studied by several workers [10,24-32].

The stomatal frequency is observed maximum on abaxial surface of leaf lamina in control plants, and while it is minimum at leaf base of adaxial surface in plants grown on heavy metal treated soils (Treatment No. I). The stomatal frequency is

observed maximum on adaxial surface of leaf apex and while it is minimum at leaf base of adaxial surface in heavy metal treated plants (Treatment No. I). The stomatal index was showed maximum at leaf base, apex, lamina and margin of the adaxial and abaxial surfaces of plants grown in control soil, when compared to other two treatments. Plants grown in heavy metal +1% $Ca(OH)_2$ treated (Treatment No. II) soils the stomatal index was showed maximum at leaf margin of the adaxial and abaxial surfaces as compared to other two treatments). Foliar stromal frequency in Solanaceae, though had been reported earlier in several species and the stomata were reported to be absent on adaxial surface of leaves in some plants [14,22,30,33].

The stomatal index is observed maximum on adaxial surface of leaf base in control plants, and while it is minimum at leaf midrib of abaxial surface in plants grown with heavy metal treated soils (Treatment No. I) TABLE 1.The trichome density was showed maximum at leaf base, midrib and lamina of the adaxial and abaxial surfaces in control plants and also in plants grown with (Treatment No. II) heavy metal +1% $Ca(OH)_2$ treated plants trichome density at leaf apex and margin of the adaxial and abaxial surfaces as compared to other treatments. The trichome density is observed maximum on abaxial surface of leaf margin in plants grown with heavy metal +1% $Ca(OH)_2$ treated soils (Treatment No. II), and while it is minimum at leaf base of adaxial surface of plants grown in heavy metal treated soils (Treatment No I). Foliar stromal frequency in Solanaceae, though had been reported earlier in several species and the stomata were reported to be absent on adaxial surface of leaves in some plants [14,22,30,33] TABLE 2.

TABLE 1. **Characteristic feature of which are same in three treatments adaxial and abaxial epidermal cell complex, stomatal cell complex and costal cell complex of *Withania somnifera* leaves grown in three treatments.**

S. No	Parameters	Shape	Anticlinal Wall	Outer wall	Cytoplasm	Surface	Orientation	Arrangement
1	Epidermal cell complex	Polygonal non-linear	Straight	Flat	Dense	Smooth	Variously	Irregularly
2	Stomatal cell complex	Anisocytic	Straight	Concave	Dense	Smooth	Variously	Irregularly
3	Costal cell complex	Polygonal linear	Straight	Flat	Dense	Smooth	Parallelly	Irregularly

TABLE 2. **Frequency of epidermal cells, Stomatal frequency, Trichomes density (per cm^2) and Stomatal index of *Withania somnifera* leaves grown in three treatments.**

Leaves parts	Parameters	Leaf Adaxial			Leaf Abaxial		
		Treatment No. I	Treatment No. II	Treatment No. III	Treatment No. I	Treatment No. II	Treatment No. III
Leaf Base	Epidermal cell Frequency/cm^2	56358.38	57803.46	55635.83	57947.97	62138.72	56213.87
	Stomatal Frequency/cm^2	12716.76	10549.13	11271.67	14595.37	13439.30	12716.76

	Stomatal Index	20.57	18.86	18.60	16.98	15.40	16.70
	Trichomes Density/cm^2	5202.31	2601.15	3757.22	6069.36	3612.71	5924.85
Leaf Apex	Epidermal cell Frequency/cm^2	55346.82	57369.94	50289.01	49132.94	59248.55	47832.36
	Stomatal Frequency/cm^2	5895.95	14739.88	13150.28	11994.21	11560.691	11849.71
	Stomatal Index	19.06	17.29	17.27	24.94	19.92	21.11
	Trichomes Density/cm^2	4046.24	3179.19	5057.80	5202.31	1878.61	5346.82
Leaf midrib	Epidermal cell Frequency/cm^2	59537.57	64739.88	57080.92	55057.80	70375.72	56647.39
	Stomatal Frequency/cm^2	13583.81	11560.69	12572.25	12283.23	10404.62	11416.18
	Stomatal Index	16.26	17.34	18.04	17.17	12.88	17.81
	Trichomes Density/cm^2	3323.69	2023.12	1878.61	3612.71	3034.68	3179.19
Leaf lamina	Epidermal cell Frequency/cm^2	60115.60	61994.21	54335.26	57803.46	59971.09	57947.97
	Stomatal Frequency/cm^2	15028.90	12861.27	13872.83	16473.98	14595.37	14161.84
	Stomatal Index	20	17.18	20.33	22.17	19.57	19.63
	Trichomes Density/cm^2	4046.24	3323.69	3612.71	4046.24	3323.69	3901.73
Leaf margin	Epidermal cell Frequency/cm^2	58959.53	53757.22	56213.87	61271.67	55635.83	58959.53
	Stomatal Frequency/cm^2	15028.90	10838.15	13150.28	16184.97	13439.30	13583.81
	Stomatal Index	20.31	16.77	18.95	22.53	18.56	18.14
	Trichomes Density/cm^2	4624.27	2601.15	5057.80	4913.29	4335.26	6936.41

Conclusion

From the above results, it is clear that the micro morphological character showed a good deal of variation among the differed a lot within the same surface and also on both the surfaces of the same leaf, which can be used in identification, adulteration and anatomy for further investigation. The micro morphological characters of *Withania somnifera* in epidermal cells were polygonal on the both surfaces, anticlinal walls were straight and smooth surface were dominant on both the surfaces, which are same in three treatments. Highest epidermal frequency is dominated at middle zone on abaxial surfaces in three treatments. It was observed anisocytic stomata are dominant on both the surface, which are same in three treatments. The (Treatment I) control plants showed high stomatal index on abaxial surface in leaf apex, lamina and margin compared to the (Treatment II) heavy metal treated plants and (Treatment III) Heavy metal +1% $Ca(OH)_2$ treated plants. Trichome density was supported maximum on abaxial surface in three treatments.

Acknowledgment

This research was supported by DST-PURSE, Research Project Ltr No. A-46/BOT//DST- PURSE/2011, New Delhi, India is greatly acknowledged. I am very thankful to Prof. B. Sashidhar Rao Coordinator, OU-DST PURSE Programme, Department of Biochemistry, Osmania University and Hyderabad for his support.

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