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RESEARCH ARTICLE

ANALYSIS OF CHLOPHYLL CONTENT IN *ADIANTUM CAPILLUS-VENERIS* L. GROWING IN DIFFERENT HABITATS IN DOON VALLEY AND NEARBY AREAS

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ABSTRACT

The present study focused on the analysis of photosynthetic pigments (Chl-a, Chl-b, chlorophylla/b ratio) of *Adiantum capillus-veneris* L. collected from five different locations of Doon valley and nearby areas. The areas include Maldevta, Shasthradhara, Majra, Patel Nagar, Biharigarh. Out of the five sites, plants collected from two sites (Maldevta, Shasthradhara) were more of natural habitats while other three (Majra, Patel Nagar, Biharigarh) were more near human habitat. The analysis of photosynthetic pigment was carried out by using chlorophyll extraction method as described by Arnon, 1949. Conclusion The highest concentration of photosynthetic pigment was found in plants which grow in moist and shady places i.e., Maldevta and Shasthradhara near water source, while the lowest value was obtained from the plants which belongs to dry and exposed areas i.e., Patel Nagar and Biharigarh. Highest Chl a content (0.16 mg/gfw) was recorded from Maldevta while lowest 0.083 mg/gfw have been reported from Majra near human habitat. Highest Chl b value (0.13 mg/gfw) was obtained from Biharigarh region whereas lowest value (0.008 mg/gfw) was found in Shasthradhara region. Chlorophyll a/b ratio is lesser in plants which grow in shady places as seen in the study that plants in Maldevta showed least value (3.7) while highest in exposed areas i.e., Shasthradhara (20). In our investigation it has been observed that chl a/b ratio is high in plants which are found in shady places in comparison to the plants which are grown in exposed areas so these changes can be attributed to disturbances in environmental factors i.e., increase in anthropogenic activities which leads to environmental stress. Thus it can be concluded that chlorophyll loss may be associated to environmental stress and the variation in total chlorophyll can be considered as a significant indicator of pollution.

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INTRODUCTION

The major light absorbing pigments in green plants are chlorophyll. They are found within the chloroplasts. They play a major role in photosynthesis, a complex biochemical pathway. The most common type of chlorophyll is Chlorophyll-a. It is dark green in colour. It is found in the core complexes of photosystems. The main leaf pigment includes chlorophyll-a, chlorophyll-b and carotenoids that are essential for photosynthesis process. Internal factors and environmental conditions may be responsible for the variation in leaf pigments (chlorophylls and carotenoids). Shaikh and Dongare (2008) reported that chlorophyll and carotenoids content changes with microclimatic conditions in *Adiantum* species. The ratio of chlorophyll-a and chlorophyll-b in terrestrial plants has been used as an indicator of response to light shade conditions. The small value of chlorophyll a/b can be considered as sensitive biomarker of pollution and environmental stress. The qualitative and quantitative analysis of pigments is facilitated by the absorbance properties of them.

Adiantum capillus-veneris is a common fern mostly found growing in shady and moist as well as in open and dry habitats in Doon valley and nearby areas.

MATERIAL AND METHODS

Collection of plants: The plants were collected from five different locations of Doon valley and nearby areas i.e., Maldevta, Shasthradhara, Majra, Patel Nagar and Biharigarh. The species were collected from natural habitat as well from human habitat.

Extraction of chlorophyll (Arnon, 1949): One gram of finely cut fresh leaves were taken and ground with 20 – 40ml of 80% acetone. It was then centrifuged at 5000 – 10000rpm for 5mins. The supernatant was transferred and the procedure was repeated till the residue becomes colourless. The absorbance of the solution was read at 645nm and 663nm against the solvent (acetone) blank.

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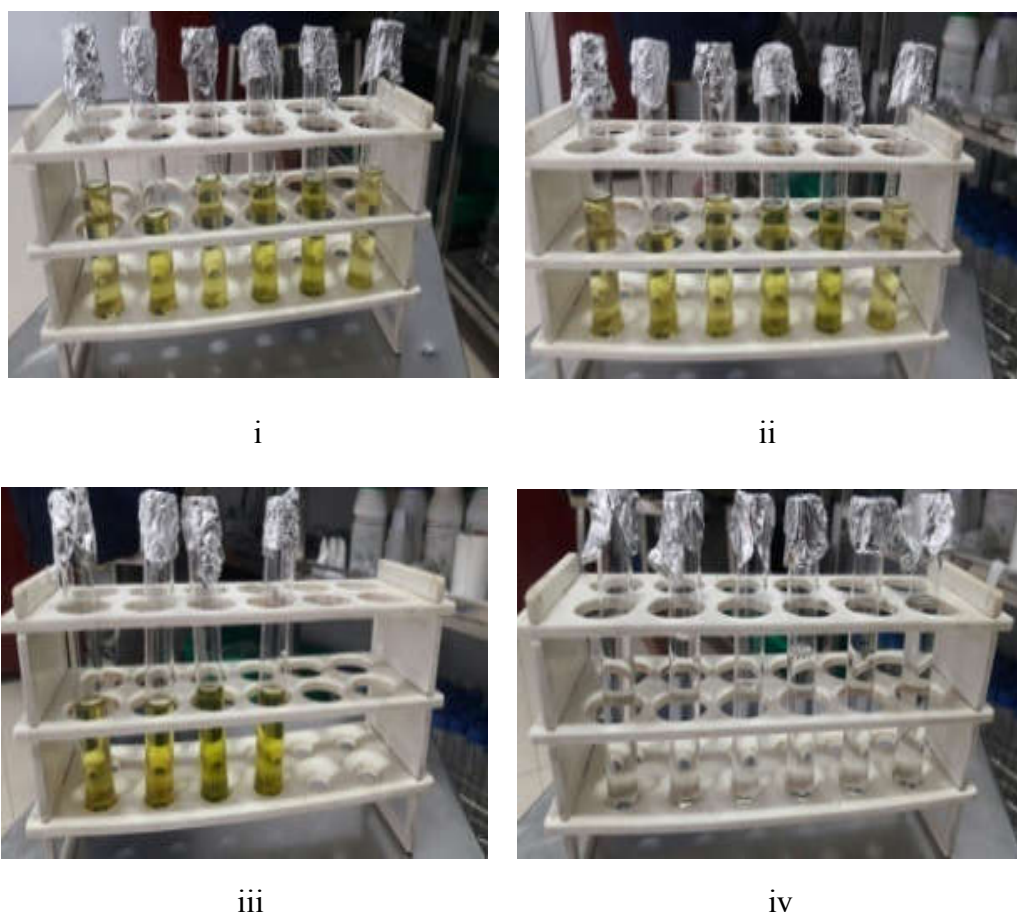


Fig. 1. Shows the extraction of Chlorophyll after 24hours to 72hours of treatment with 80% acetone

Estimation of Chlorophyll content: The concentrations of chlorophyll a, chlorophyll b and total chlorophyll were calculated using the following equation:

mg chlorophyll a /gm Fr.wt $[12.7 (A_{663}) - 2.69(A_{645})] \times V/1000 \times W$

mg chlorophyll b /gm Fr.wt $[22.9(A_{645}) - 4.68 (A_{663})] \times V/1000 \times W$

Total chlorophyll (mg/gfw) $= 20.2(A_{645}) + 8.03(A_{663}) \times V/1000 \times W$

Where,

A = Absorbance of chlorophyll extract at the specific indicated wavelength,

V = Final volume of the solution & W = gm of tissue extracted

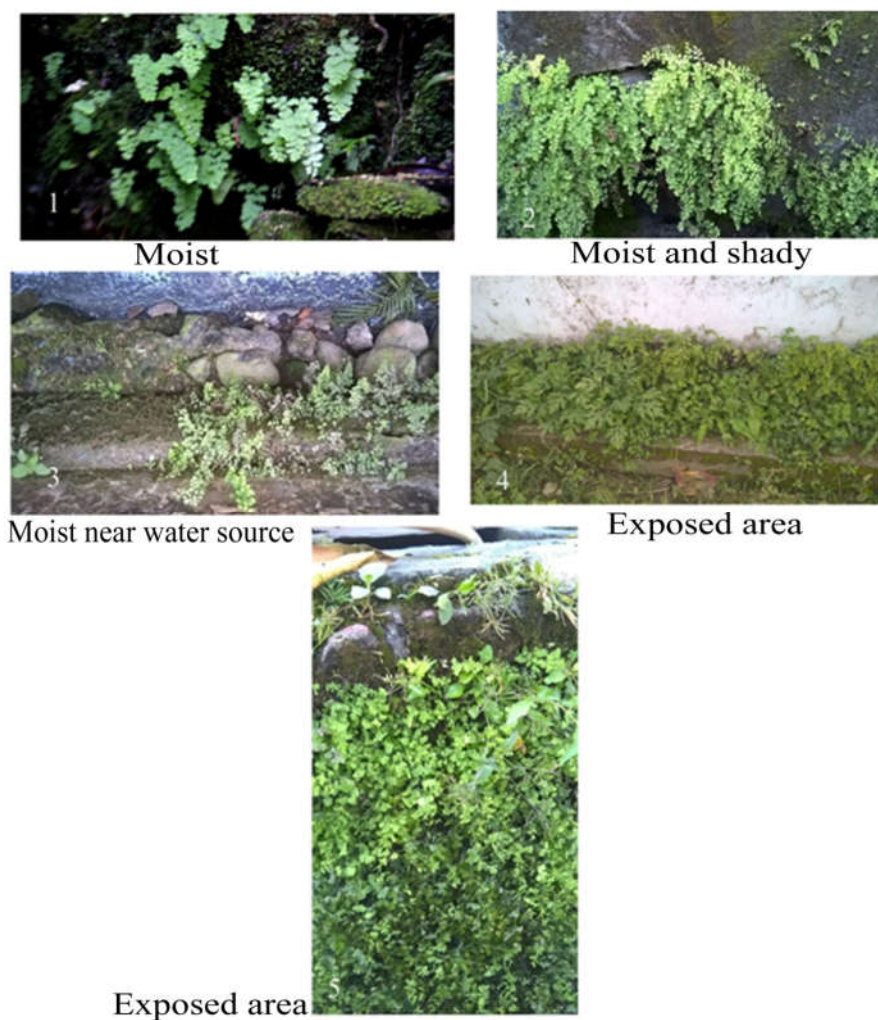
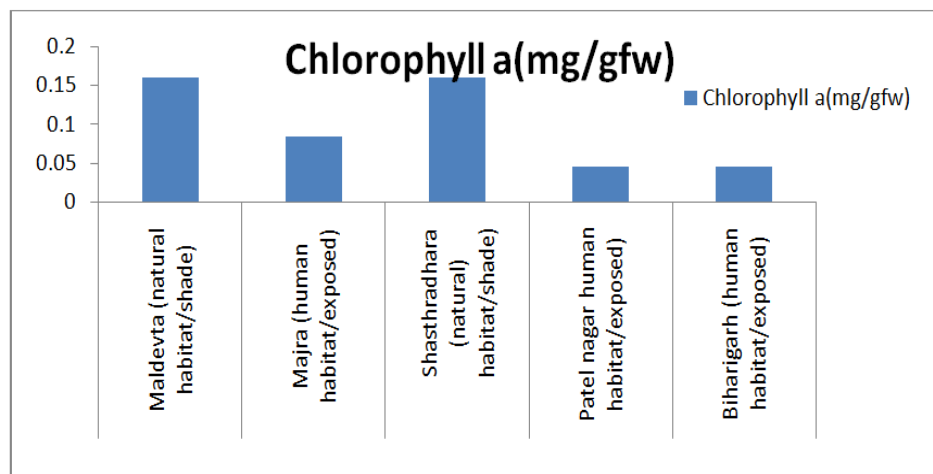
RESULT AND DISCUSSION

Majority of the ferns are found growing in shady, moist conditions, damp areas. Doon valley which is situated up to elevation of 1500 m with tropical and subtropical vegetation dominated by Sal forest (Chhaya *et.al.* 2017). *Adiantum* species is mostly found in exposed areas of forest with scanty vegetation especially *Adiantum capillus-veneris*. (Chhaya *et.al.* 2013). Fig 1 shows the distribution of *Adiantum* species at various places: Estimation of Chlorophyll was done using fresh green fronds extracted with 80% acetone solvent. The absorbance values of chlorophyll extracts were measured at two different wavelengths 645nm and 663nm respectively. The calculations of the amount of chlorophyll a, chlorophyll b, chlorophyll a/b and total chlorophyll were based on the absorbance values obtained, using Arnon's (1949) equation

and were tabulated in table 1. Extraction of Chlorophyll can be observed in Fig 1. As Blank (i), after 24 hour (ii), after 48hours (iii) and after 72hours (iv). It was observed from the present investigation that highest Chlorophyll a concentration (0.16mg/gfw) was reported in Maldevta and Sahastradhara locations and least (0.083mg/gfw) in Majra (Fig: 3). Highest chlorophyll b values (0.13mg/gfw) were showed by plants belonging to Biharigarh location and least were (0.008mg/gfw) observed in the case of sahatradhara. The chlorophyll contents were greater in the shade ferns when compared to the sun ferns (Boyce & Mohamed 1987). The greater values of total chlorophyll were recorded from the region of Maldevta (0.204 mg/gfw) and Sahatradhara (0.205 mg/gfw) whereas lower in Patel nagar and Biharigarh (0.058 mg/gfw). Shaikh & Dongare(2008) showed that variation in chlorophyll and carotenoids contents varied with microclimatic conditions in *Adiantum philippense* L. Because of numerous factors concerned with nutrition and light which can influence chlorophyll content, and because the plants sampled were growing under a variety of conditions (Wolf 1958). The variation in total chlorophyll and its loss is believed to be associated with environment stress and good indicator of stress which is indicated by observed ratio of chlorophylla/b. According to Lahani *et.al.* (2003) chlorophyll a/b ratio is lesser in the plants that grow under the shade than in plants that were exposed to full sunlight. But in our study deviation has been observed as the highest chlorophyll a/b ratio (20 mg/gfw) in case of Shasthradhara (Shady place). The reason for this variation may be the environmental stress due to increase in anthropogenic activities as it a tourist spot.

Table 1. Estimation of Chlorophyll in *Adiantum capillus-veneris* of different locations in Doon Valley

S. no	Name of the plant	Habitat	Chlorophyll a (mg/gfw)	Chlorophyll b (mg/gfw)	Total Chlorophyll (mg/gfw)	Chlorophyll a/b (mg/gfw)
1.	<i>Adiantum capillus-veneris</i>	Maldevta (natural habitat/shade)	0.16	0.043	0.204	3.7
2	<i>Adiantum capillus-veneris</i>	Majra (human habitat/exposed)	0.083	0.021	0.105	3.9
3	<i>Adiantum capillus-veneris</i>	Shasthradhara (natural) habitat/shade)	0.16	0.008	0.205	20
4	<i>Adiantum capillus-veneris</i>	Patel nagar human habitat/exposed)	0.045	0.012	0.058	3.7
5	<i>Adiantum capillus-veneris</i>	Biharigarh (human habitat/exposed)	0.045	0.13	0.058	3.7

**Fig. 2. 1.shashtradhara 2. Maldevta 3.Majra 4. Biharigarh 5. Patel nagar****Fig. 3. Graphical representation of chlorophyll a content estimated in plants of different locations at Doon valley**

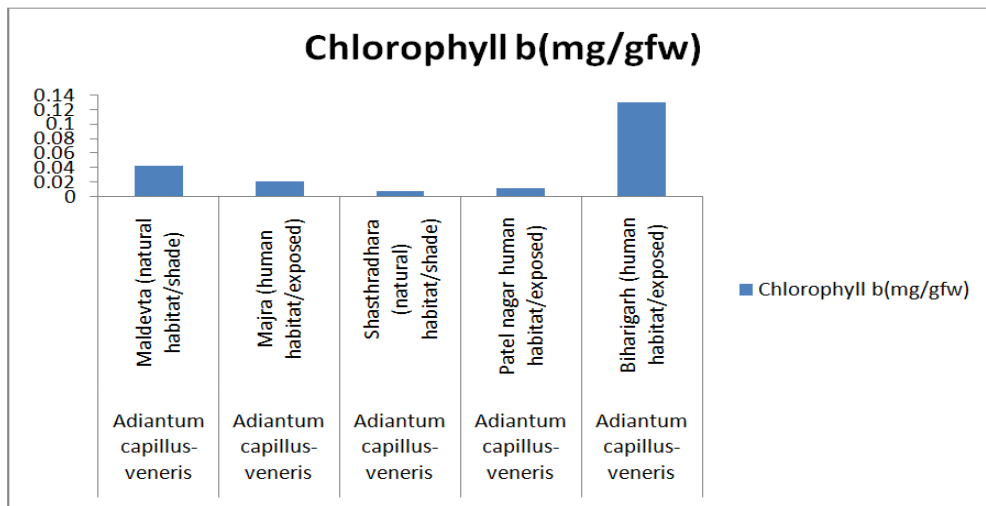


Fig. 4. Graphical representation of chlorophyll b content estimated in plants of different locations at Doon valley

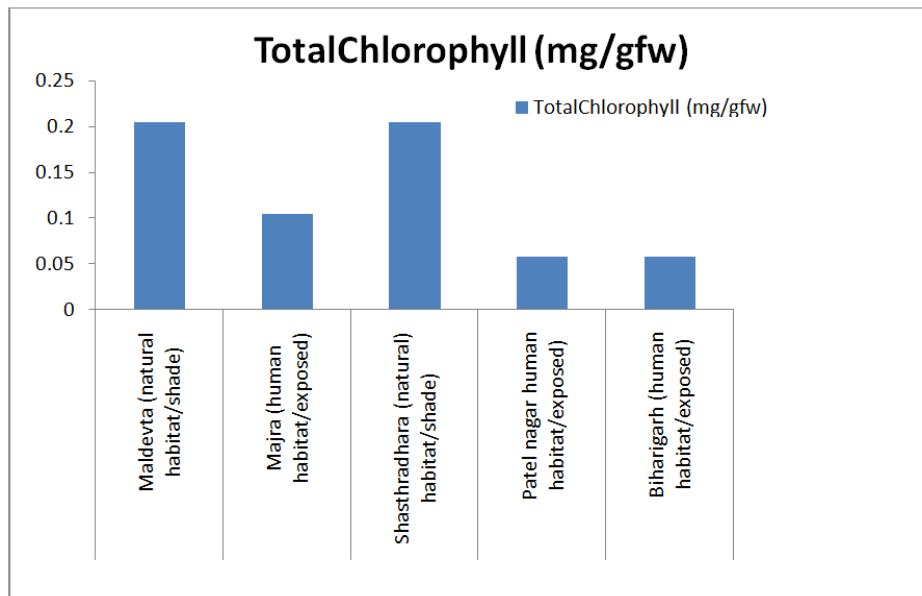


Fig. 5. Graphical representation of Total chlorophyll content estimated in plants of different locations at Doon valley

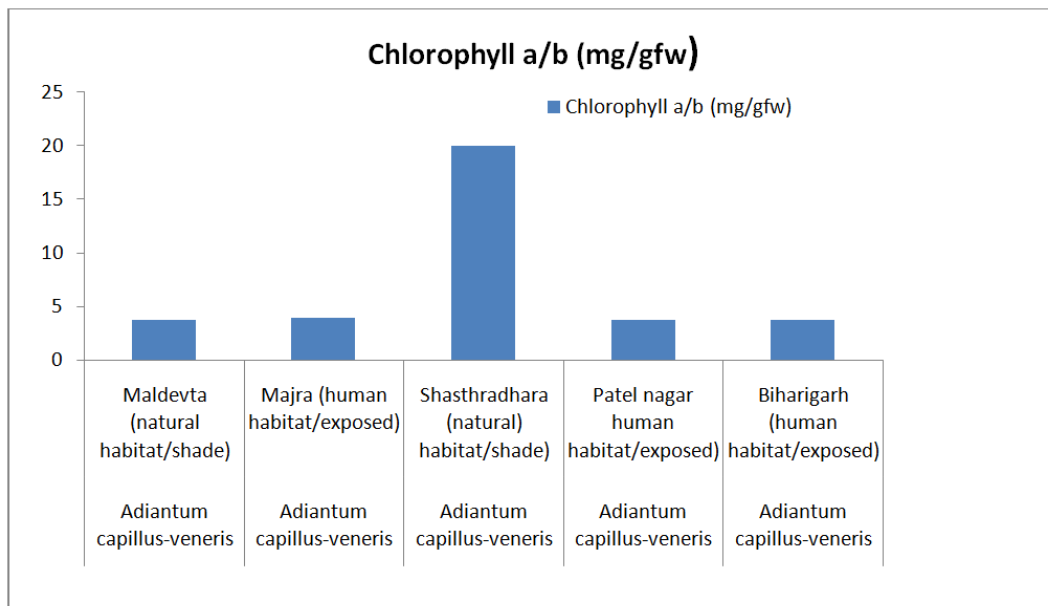


Fig. 6. Graphical representation of chlorophyll a/b ratio estimated in plants of different locations at Doon valley

Environmental Pollutant can easily interact with essential plant metabolic processes leading to defect in Chl a and b production or function and leading to impairment in whole photosynthetic activity & as a result affecting plant fitness. Low concentrations of chlorophyll can directly limit photosynthesis and hence growth and yield. The *Adiantum capillus-veneris* L. collected from the moist places were having large and thick leaves as compared to those growing near human habitat as also seen in Fig 2.

Conclusion

From the present study it can be concluded that variation in chlorophyll can be observed in ferns of same species from shady places to moist and damp places. Since the habitat where ferns are reported are more under threat due to human stress may be habitat fragmentation, human interference in the forest area etc. The major factor responsible is environmental pollution which has deleterious effects on the production of chlorophyll. Further study has to be undertaken by analysis of different factor such as plant biomass of plant collected from different source, variation in the size of the leaves, changes in the stomatal structure etc. As it is well known that chlorophyll or chlorophyll derivatives can be utilized as photodynamic agent in Tumor and cancer therapy. (Brandis et al., 2006) this aspect can also be experimentally carried out in case of *Adiantum capillus-veneris*.

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