INTRODUCTION

Mangroves create a unique ecological environment, having a rich assemblage of various species. It has many peculiar features than other terrestrial plants. However, mangrove species are classified into true or exclusive mangroves or strict or obligate mangroves, and Mangrove associate or nonexclusive or semi mangroves (1). A true or exclusive Mangrove occurs only in mangrove environment and not extends into terrestrial habitat. In addition to this morphological specialization such as profuse lateral roots, exposed aerial roots, viviparous, water dispersed propagules and physiological mechanism for salt excretion or salt exclusion are also observed. Non exclusive species or mangrove associates are mainly distributed in terrestrial or aquatic habitat but also occur in the mangrove ecosystem (1,2). Of the 14 districts of Kerala, mangroves are spread over about in ten districts of which majority occur in the northern region. Kannur has highest area of mangroves (755Ha) followed by Kozhikode (293Ha) and Ernakulam (260Ha) (3).

In green plants, photosynthesis takes place in the chloropll containing thylakoid membrane of the chloroplast. Chlorophyll a is the primary photosynthetic pigment which captures light with narrow and specific visible range of sunlight. Chlorophyll b and carotenoids act as accessory supportive photosynthetic pigments and they accept light of wide range (4). For mangroves, the concentrations of leaf pigment can be associated with environmental factors such as ambient temperature/sunlight (5), water availability and salinity (6). Chlorophylls are the most important leaf pigment responsible for photosynthesis (7) and relative chlorophyll content has positive relationship with photosynthetic rate (8).

1. INTRODUCTION

Mangroves are trees inhabiting the intertidal zones of tropical and subtropical coast. They are classified as true mangroves and mangrove associates. In the present study leaf samples of various age groups from five mangrove species and six mangrove associated species were selected for the estimation of chlorophyll content. True mangroves showed comparatively high amount of chlorophyll than mangrove associates and moreover in medium aged leaves chlorophyll contents were more than young and old leaves.

Keywords: Mangrove, mangrove associate, chlorophyll.

2. MATERIALS AND METHODS

2.1. Plant material

For the present study, young, medium and old leaves were collected randomly from about five mangroves and six mangrove associated species from Payangadi, Kannur, Kerala. Leaves of three developmental stages were selected considering the colour variation, leaf size and their position on the stem from apex to base direction, ie; leaves from the young tip/apex of the stem as juvenile/ young leaves and so on, for comparing the relative chlorophyll content. The selected mangrove plants are Rhizophora mucronata Poir, Avicennia marina (Forssk.) Vierh., Avicennia officinalis L., Acanthus ilicifolius var. subinteger Nees, and Bruguiera cylindrica (L) Blume. The selected mangrove associated plants are; Ipomoea macrantha Roem. & Schult., Cayratia trifolia (L) Domin., Derris trifoliata Lour. Premna serratifolia L., Clerodendrum inerme (L) Gaertn. and Cyperus sp. The leaves were collected in poly bags and were immediately brought into the laboratory for biochemical analysis.

2.2. Quantitative estimation of chlorophyll

100 mg. fresh leaf tissues were homogenized with 80% acetone. The extract was centrifuged for five minutes and the supernatant was collected. The residue was re extracted with 80% acetone and centrifuged. The process was repeated till the pellet become colorless. The final volume of the combined supernatant was noted. The absorbance of the extract was noted at 663nm and 645nm. using UV-Visible spectrophotometer. The total chlorophyll, chlorophyll a, chlorophyll b, chlorophyll a/ chlorophyll b ratio were calculated using the formula suggested by Arnon (9).

Chlorophyll expressed as mg/g fresh tissue.
Chlorophyll a = 12.7(A_{663}) - 2.69(A_{645}) * V/1000*W.
Chlorophyll b = 22.9(A_{645}) - 4.68(A_{663}) * V/1000*W.
Total Chlorophyll = 20.2(A_{645}) + 8.02(A_{663}) * V/1000*W.

A = Absorbance at specific wave length
V = Final volume of chlorophyll extract in 80% acetone.
W = Fresh weight of tissue extracted.

3. RESULTS AND DISCUSSION

The present experiment on quantitative estimation chlorophyll a and b, total chlorophyll, the ratio of chl.a/b for selected mangroves and associated plant species, (Table 1) showed little differences between mangroves and associated plant species. In the study, it is estimated that the old leaves of *Avicennia marina* contained highest amount of chlorophyl a and chlorophyll b (1.37,1.2 mg/g fresh tissue respectively) where as young leaves of *Acanthus ilicifolius* showed the lowest amount (0.23mg/g fresh tissue - chlorophyll a and 0.13 mg/g fresh tissue - chlorophyll b). Considering the mangrove associated plants, young leaves of *Derris trifoliata* showed highest amount of chlorophyll a (1.07 mg/g fresh tissue) and intermediate or medium aged leaves have shown increased amount of chlorophyll b (1.44 mg/g fresh tissue). Medium aged and old aged leaves of *Cayratia trifolia* showed the lowest amount of chlorophyll a (0.39 mg/g fresh tissue) and chlorophyll b (0.18 mg/g fresh tissue). Leaf age and physiological state are important determinant of the chlorophyll content (10).

| Plant | Plant age | Chl. a | Chl. b | Tot. Chl. | Chl. a/b |
|-------|-----------|--------|--------|----------|----------|
| Ao    | Y         | 0.57   | 0.27   | 0.84     | 2.11     |
|       | M         | 0.82   | 0.7    | 1.52     | 1.17     |
|       | O         | 0.83   | 0.33   | 1.16     | 2.52     |
|       | Y         | 1.12   | 0.63   | 1.75     | 1.78     |
| Am    | M         | 1.23   | 0.71   | 1.94     | 1.73     |
|       | O         | 1.37   | 1.2    | 2.57     | 1.14     |
|       | Y         | 0.5    | 0.26   | 0.76     | 1.92     |
| Rm    | M         | 0.74   | 0.37   | 1.11     | 2.00     |
|       | O         | 0.87   | 0.3    | 1.17     | 2.90     |
|       | Y         | 0.23   | 0.13   | 0.36     | 1.77     |
| Ai    | M         | 0.32   | 0.18   | 0.5      | 1.78     |
|       | O         | 0.25   | 0.17   | 0.42     | 1.47     |
|       | Y         | 0.81   | 0.29   | 1.1      | 2.79     |
| Bc    | M         | 0.77   | 0.9    | 1.67     | 0.86     |
|       | O         | 0.88   | 0.5    | 1.38     | 1.76     |
| Iv    | M         | 0.45   | 0.72   | 1.17     | 0.63     |
|       | O         | 0.51   | 0.68   | 1.19     | 0.75     |
|       | Y         | 1.07   | 1.24   | 2.31     | 0.86     |
| Dt    | M         | 0.78   | 1.44   | 2.22     | 0.54     |
|       | O         | 0.88   | 0.67   | 1.55     | 1.31     |
|       | Y         | 0.61   | 0.3    | 0.91     | 2.03     |
| Ps    | M         | 0.71   | 0.33   | 1.04     | 2.15     |
|       | O         | 0.8    | 0.35   | 1.15     | 2.29     |
|       | Y         | 0.59   | 0.32   | 0.91     | 1.84     |
| Ct    | M         | 0.39   | 0.41   | 0.8      | 0.95     |
|       | O         | 0.44   | 0.18   | 0.62     | 2.44     |
|       | Y         | 0.47   | 0.18   | 0.65     | 2.61     |
| Ci    | M         | 0.57   | 0.25   | 0.82     | 2.28     |
|       | O         | 0.57   | 0.24   | 0.81     | 2.38     |
|       | Y         | 0.84   | 0.31   | 1.15     | 2.71     |
| Cy.   | M         | 0.81   | 0.34   | 1.15     | 2.38     |
|       | O         | 0.65   | 0.37   | 1.02     | 1.76     |

Table1. Estimation of chlorophyll for different mangroves and associated plant species.

Fig. 1. Showing chlorophyll a, chlorophyll b, total chlorophyll and chlorophyll a/b ratio of mangroves.

Fig. 2. Showing chlorophyll a, chlorophyll b, total chlorophyll and chlorophyll a/b ratio of mangrove associates.
The chlorophyll content in the leaves depend on the endogenous factors such as pigment synthesis and degradation and stage of leaf development. In addition, environmental factors like shade, light, temperature, drought, water logging, soil salinity etc affect the chlorophyll content. Generally chl.a/b ratio of mangroves and associated plant species was higher in p growing in non saline soil than saline soil (11). The reduction in the chlA/b ratio which is an adaptation to unfavorable conditions may be due to the fact that chlorophyll b is more resistant for degradation than chlorophyll a (12). The total chlorophyll content in the mangrove plants Bruguiera gymnorrhiza, Excoecaria agallocha and Heritiera fomes grown in nonsaline condition was higher than that of in saline condition (13). Increased count is an indication of increasing rate of assimilation thus enhancement in the rate energy transfer and production. In the present study an increase in the total chlorophyll content is observed in old leaves of Avicennia marina (2.57 mg/g fresh tissue) and young leaves of Derris trifoliata (2.31 mg/g fresh tissue).

Seasonal change in the chlorophyll a content is observed (14), where during rainy season an increase in chlorophyll a content in mangroves than that of dry seasons. It is observed that during winter an increase in total chlorophyll and the highest amount of carotenoids in summer reflect an adaptation to enhance the photo protection properties of mangrove leaves (4). An increase in the chlorophyll a and b in the leaves under shaded, receiving diffused light is observed than the leaves under direct sun light (15). Simultaneously analysis of leaves of different age group shows that leaves with intermediate or medium age comparatively have the highest content of chlorophyll a and b; these results also well support present study which also indicates that intermediate leaves are more productive than the remaining aged leaves. In general, true mangroves show comparatively more chlorophyll content than associated plant species.

4. CONCLUSION

The result of the work adds to our understanding of the relationship of leaf age and its physiological state to the chlorophyll content. Low chlorophyll concentration may also indicates plant physiological stress. Since chlorophyll take part in the conversion of solar energy in to chemical energy, their level in the leaf tissue is one of the important features governing photosynthetic efficiency of plants. Thus it can be concluded that in relation to ecophysiological adaptation understanding of the nature of the pigments in mangroves and their associated plants is a parameter for their conservation and propagation under different salinity condition.

REFERENCES

1. Tomlison, P.B. (1986). The Botany of Mangroves. Cambridge University Pres. Cambridge, U.K. p. 413.
2. Lacedra, L.D. (1998). Trace metals biogeochemistry and diffuse pollution in mangrove ecosystems. ISME Mangrove Ecosystems Occasional. 2: 1-16.
3. Basha, S.C. (1991). Distribution of mangroves in Kerala. Indian For. 117: 437-449.
4. Padma, M., N. Lele, R.N. Samal, T.V.R. Murthy, A.K. Patnaik and S. Nanda, (2017). Diversity, phenology and variation of seasonal leaf photosynthetic pigments in mangroves of Chilka Lagoon (Odisha). India. Bio. Sci. Dis. 8(2): 211-219.
5. Saenger, P. (2002). Mangrove ecology, silviculture and conservation. Kluwer Academic Publishers, Dordrecht.
6. Lacedra, L.D. (2002). Mangrove ecosystems: function and management. Springer, Berlin.
7. Pastor-Guzman, J., P.M. Atkinso, J. Dash and R. Rioja-Nieto, (2015). Spatiotemporal variation in mangrove chlorophyll concentration using landsat 8. Remote Sens. 7: 14530-14558.
8. Anjum, S.A., Xiao-yu-Xie, W. Long-Chang, M.F. Saleem, M. Chen and Wang Lei, (2011). Morphological, physiological and biochemical response of plants to drought stress. Afr. J. Agric. Res. 6(9): 2026-2032.
9. Arnon, D.J. (1949). Copper enzymes in isolated chloroplast polyphenoloxidase in Beta vulgaris. Plant Physiol. 24: 1-5.
10. Patrik, D. (2007). Evaluating a chlorophyll content meter on three coastal wetland plant species. J. Agrl. Fo. Env. Sci. 1: 1-11.
11. Patwardhan, M.M. and P.D. Chavan, (2013). Ecophysiological studies of Pongamia pinnata and Canavalia cathartica growing in saline and nonsaline habitat from Ratnagiri district of Maharashtra. Proceedings of UGC sponsored national level seminar Dynamics of Mangrove Ecosystem. November 29-30, 2013. Organized by Hindi Vidya Prachar Samti, Ramniranjan Jhunjhunwala College, Ghatkopar in association with vanashakti and ATBS.
12. Young, A.J. (1993). Factors that affect the carotenoid composition of higher plants and algae In: Carotenoids in photosynthesis (eds.) by Andrew Young and George Britton. Springer Science+Business Media.
13. Pramita, N.D., D. Nirjhar and D. Sauren, (2009). Differential expression of physiological and biochemical characters of some Indian mangroves towards salt tolerance. Physiol. Mol. biol. Plants. 15: 151-160.
14. Flores-de-Santiago, F., M.K. John and F. Flores-Verugo, (2012). Seasonal changes in leaf chlorophyll a content and morphology in a subtropical mangrove forest of Mexican Pacific. Mar. Ecol. Prog. Ser. 444: 57-68.
15. Prayaga, M.P. and G.M.N. Rao, (2014). Determination of chlorophyll a and b of some mangrove species of Vishakhapatnam, Andrapradesh, India. Inter. J. Phytother. 4: 131-132.