CHAPTER - II

MATERIALS
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MATERIALS AND METHODS

Terminalia plant description

*Terminalia arjuna* Class: Dicotylednes, Sub class: Polypetale, Series: Calacyflorae, Order: Myrtales, Family: Combartiaceae. Terminalia plant is Evergreen, handsome tree, up to 30 m tall; often buttressed; crown spreading; outer bark stiff, flaking - off in pieces, inner bark white and smooth outside, coloured when cut, exuding red resin, wood brown, variegated with darker streaks, leaves thick coriaaceous, oblong or oblanceolate, 7-11 x 3.4-5cm, glabrous, apex obtuse, margin crenateserrate, base obtuse subcordate; secondary nerves 14-16 pairs, parallel; petiole to 1.5cm, 12 glands on the petiole, close to the base of lamina. Flowers dull yellow, spikes axillaries in panicles. Drupe dark brown, 2-4 cm long, obvoid-oblong, dark, woody, fibrous, apex notched, wings 5, equal, projecting. striated with numerous curved lines. *Terminalia arjuna* growth along streams, river banks, ravines and dry water courses; reaching a large size on fertile alluvial loam up to 1300 m. The tree is a moderate shad-bearer and does not tolerate dense overhead shade. It has more or less superficial root system which radially along the banks of streams forming its favourable habitats.

Treatment of Terminalia leaves

The study begins with by procuring the *Terminalia arjuna* from the R S R S (Regional Sericulture Research Station), Anantapur, India. The experiment was conducted in leaf of 45 days old. They detached
from the plants and washed in deionized water and were surface sterilized with 0.1 per cent mercuric chloride solution for 30 seconds, then washed with distilled water. The leaf bits were placed in petridishes of 20 cm diameter, containing distilled water or fluoride solutions. 3 leaf bits were kept in each petridish. Fluoride is available in many salts forms like, sodium fluoride, Calcium fluoride, aluminum fluoride etc. Sodium fluoride with molecular formula weight 41.988 is used in the investigation to study the effects of fluoride on Terminalia leaf.

**Invitro foliar symptoms**

The samples were showed under microscope. Different concentration i.e. 100 ppm, 200 ppm and 300 ppm solutions of the above salts were prepared in distilled water and distilled water was alone served as control. Three petridishes were placed for each concentration of fluoride. Petri dishes were kept under a light intensity of approximately 150 wm-2 and temperature of 27°C ± 3°C the solutions were replaced every day with fresh once. The samples, were estimated after, for morphological and biochemical studies, (2, 4, 6 and 8 days) 48, 96, 144 and 192 hrs of incubation.

**Estimation of Total Chlorophyll Content (TCC)**

The Total chlorophyll content was estimated in the leaves of control and 5, 10, and 15 days water stressed plants of both varieties according to the method of Arnon (1949). Fresh leaves were taken, washed and blotted to dry and the leaf material was homogenized in a
prechilled mortar using cold 80% acetone. All the supernatant collected at each time were pooled and made to known volume with 80% cold acetone. The optical density of the acetone extract was measured at 645nm and 663nm using 80% acetone as blank in a spectrophotometer (Bausch and Lomb, Spectronic 88). Total chlorophyll content was calculated by employing the following formula. 

\[ \text{TCC} = 20.2 \times \text{O.D. (at 645 nm)} + 8.02 \times \text{O.D. (at 663 nm)} \]

**Estimation of Photosynthesis rate**

Rate of photosynthesis was monitored by using a portable photosynthesis system (Infrared gas analyzer: LCA -3) equipped with a parkinson leaf chamber (6.1 cm$^2$), (PLC) (Analytical Development Co., Hoddesdon, U.K). The measurements were made between 8am to 10 am at photosynthetic photon flux density of approximately $1100 \pm 100 \mu \text{mol m}^{-2} \text{S}^{-1}$. The leaf temperature was ranged between $30^\circ \text{C} \pm 20^\circ \text{C}$. The measurements were done in the third leaf (fully expanded) from the top, since the leaf was found to posses, maximum photosynthetic capacity. The selection of third leaf from top in terminalia for eco- physiological studies had also been suggested by Chen (1992).

C-index was obtained from the formula as by Vadell and Medrano (1992). 

\[ \text{C-index} = 1(\text{Ci}/\text{Ca}) \]

Where,

Ci and Ca are intercellular and ambient CO$_2$ concentrations respectively

Air CO$_2$ concentration $340 \pm 11^{-1}$. 
Estimation of antioxidative enzymes

Catalase

Catalase activity was assayed and estimated as per the method of Barber (1980). The reaction mixture consisted enzyme extract, 0.005M H2O2 and 0.05M Tri Buffer, pH 7.0. After incubation it for 1 min at 25° C, the reaction was stopped by adding 1.0 ml of 2.5 N H2SO4. The residual H2O2 was titrated with 0.01N KMNO4. A blank was maintained with the reaction mixture at Zero time. Catalase activity was expressed as mg H2O2 Oxidized per gram fresh weight per min.

Guaiacol Peroxidase

Guaiacol peroxidase (EC 1.11.1.7) was assayed according to Egley et al., (1983). Fresh root / shoot samples weighing 200mg were homogenized in 5 ml of cold 50 mM Na -Phosphate at 22000 x g 10 min and the dialised enzyme extract were used for the assay. Assay mixture in a total volume of 5 ml contained 40 mM Na-phosphate buffer (pH 6.1) 2mM H2O2, 9 mM Guaiacol and 50 μ 1 enzyme. Increase in a absorbance was measured at 420 nm (Extinction co-efficient of 26.6 mM⁻¹ Cm⁻¹) at 30s intervals up to 2 min, using a Bausch and Lomb spectronic -20 spectro photo meter (USA). Enzymes specific activity is expressed as μmole of H2O2 reduced min⁻¹ (mg protein⁻¹).

Ascorbate Peroxidase

Above 200mg leaf material sample were homoginated in 5 ml of 50 mM K Phosphate Buffer (pH 7.8) containing 1% PVP, 1 mM Ascorbic
Acid and 1 mM PMSF as described by Moran et al., (1994) after centrifugation at 22000 x g for 10 min at 40° C the supernatant against was dialised against the same extraction buffer and it served as enzyme. Ascorbate Peroxidase was assayed according to Nakon and Asada(1981) reaction mixture in a total volume of 1 ml contained 50 mM K-Phosphate Buffer (pH7.0) 0.2 mM Ascorbic Acid 0.2 mM EDTA, 20 μM H₂O₂ and enzyme was the last comported to be added and decrease in absorbance was recorded at 290nm (extinction of 2.8 mM⁻¹ Cm⁻¹ using a UV-V is spectrophotometer at 30s intervals up to 7min. Correction was made for the low. Non enzymic oxidation of Ascorbic Acid by H₂O₂. The specific activity of enzyme is expressed as μ mol ascorbate oxidized min⁻¹ (mg protein⁻¹).

Statistical Analysis

All the requirements obtained in this investigation were subjected to statistical analysis. The date obtained for each parameter was analysed for their significance according to the method of Duncan's multiple ranger test (Duncan, 1955). The significance was calculated at 5% level (p < 0.05).