CHAPTER THREE

STUDIES ON PHOTOSYNTHESIS : A PROCESS OF SOLAR TO BIOMASS CONVERSION

CHAPTER - III

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3.1 INTRODUCTION :

Solar energy conversion by green plants supplies vertually all the energy for leaving cells on the earth. Fossil fuels, on which we depend for a large part of our energy requirement, are basically the products of plant biomass. The principal process, which converts solar energy into stored chemical energy in biomass is 'Photosynthesis' (Beadle 1985, Govindjee 1983, Fong 1982 and Kamen 1963). There are various methods to study the photosynthesis and one of them is the study of photosynthesis by physicochemical method. In the present investigation, we have designed and fabricated the photosynthetic set up with the help of our workshop and studied the effect of light intensity on photosynthesis of Leucaena leucocephala.

3.2 THEORETICAL BACKGROUND :

3.2.1 Photosynthesis Process :

The photosynthesis in green plants is the process in which sunlight is captured by pigment materials and through a sequence of primary and secondary reaction initiated by the excited chlorophyll reaction centres, converted into stored chemical energy (Fong 1982). At the physico-chemical level this happens through the reduction of CO_2 to triose phosphate. (Fig. 3.1).

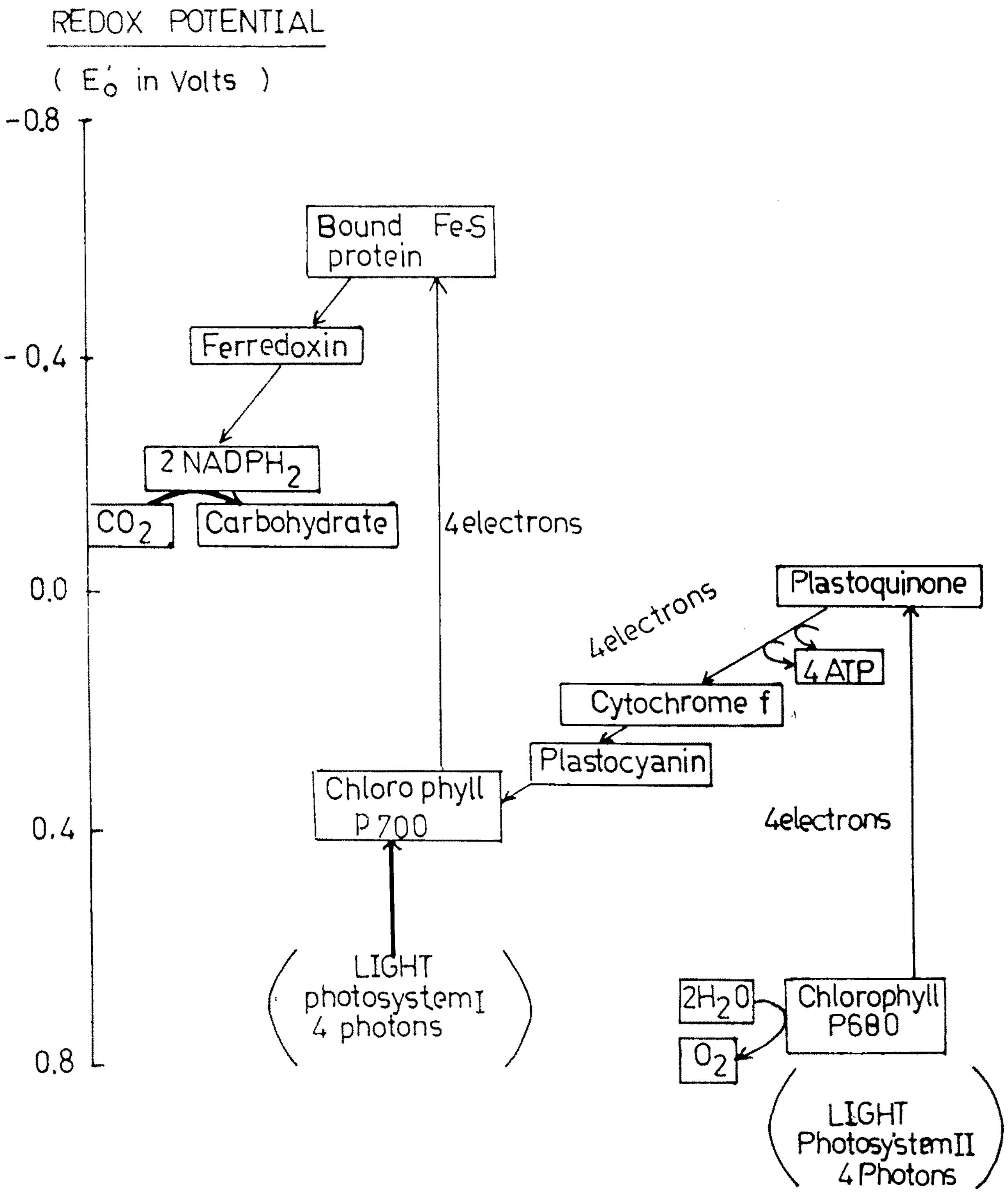

Fig. 3.1 : Photosynthetic electron transport scheme

The overall process can be summarised as the transfer of hydrogen atoms from water to carbon dioxide to form carbohydrate (biomass) with liberation of oxygen.

$$nH_2O + nCO_2 \xrightarrow[\text{plant}]{\text{solar energy}} \underset{|(CH_2O)_n|}{\text{Biomass}} + nO_2 \quad \ldots (1)$$

In short the above process can be written as,

solar energy —— plant ——— fixed chemical energy ... (2)

3.2.2 Energetics of Photosynthesis :

Energy storage in photosynthesis occurs by formation of 0-0 bonds (-58 K.cal/mole) and by destruction of O-H (-11 K cal/mole) and C-O bonds (-95 K cal/mole) which is an endothermic reaction. These energetics of the different photosynthetic step may be summarised as below :

1) In green plants, photosynthesis is governed by chloroplast. The chloroplast is subdivided into 300 pigment molecules and a single reaction centre. These pigment molecules (carotenoids, phycoerythrum, phycocyamin and various chlorophylls) absorb light in the range 400 to 700 nm. of wavelength. The pigment molecules after light absorption, become highly energiged and unstable. They give out the excitation energy for chemical stored energy products for use in later reaction.

2) In second step i.e. photoxidation means ejection of electrons from water molecules to produce H^+ ions needed for CO_2 reduction. Thus photosynthetic reaction can be considered as an electrochemical oxidation reduction reaction (Fig. 3.1).

$$2H_2O \xrightarrow{\text{light}} O_2 + 4H^+ + 4e^- \quad E_o = +0.81 \text{ volts} \quad \ldots (3)$$

$$CO_2 + 4H^+ + 4e^- \xrightarrow{\text{park}} CH_2O + H_2O \quad E_o = -0.40 \text{ volts} \quad \ldots (4)$$

where E_o is the standard electrochemical potential of the given reaction as normal hydrogen electrode (NHE) scale. During this reaction, the biological energy packet ATP (Adinoceine tri phosphate) and strong reductant NADPH (Nicotinamide Adinoceine Dinucleotide phosphate) are also obtained as triproduct besides the main product H^+

3) The third step involves the final reduction of CO_2 in dark. Sugar is finally synthesised from atmospheric CO_2 and the light reaction products ATP and NADPH in the following stiochiometry.

$$6\ CO_2 + 18\ ATP + 12\ NADPH + 12\ H^+ \longrightarrow C_6H_{12}O_6 + \\ + 18\ ATP + 12\ NADP + 6\ H_2O \quad \ldots (5)$$

where NADPH and NADP are reduced and oxidised Nicotinamide dinucleotide phosphate.

Thus the potential required to drive overall reaction is 1.21 volts, free energy change of the photosynthetic reaction is 110 K.cal as obtained by the formula

$$G = n.F.E. \quad \ldots (6)$$

where, n = number of electron involved in the reaction

F = Faraday constant (96500 Cooloumbs) and

E = Potential required to drive the reaction.

On electronic energy scale, volts can be treated as electron volvs (eV). Thus the incident light should have energy at least 1.21 eV to carry out the photosynthetic reaction. It is interesting to observe that even the lowest energy light ($\lambda = 700$ nm) has the energy of 1.76 eV as obtained by the formula

$$E_{eV} = 1235/\lambda \text{ (nm)} \qquad \dots (7)$$

This energy is sufficient to carry out the photooxidation even after considering some inherent lose process.

3.2.3 Energy Balance and Photosynthesis :

In previous chapter we have discussed at length the systematic studies on 'Energy Plantation' programme for biomass production. In the present chapter, we are studying the basic process of conversion of solar energy into fixed chemical energy with the help of photosynthesis. In photosynthesis process, the utilization of solar energy by the plants is only about 0.1 % and fixed carbon consumption in the form of nutrient energy by 4×10^9 people is only about 0.5 %. This production of fixed carbon is however, ten times the present world consumption of energy.

The world energy balance and photosynthesis is shown in Fig.3.2 From Figure it is seen that the scope for increasing the total utilization of solar energy into fixed chemical energy using photosynthesis is enormous For this it is necessary to examin the factors limiting conversion efficiency in plants, ultimately what efficiency we might achieve and define the conditions necessary for achieving it.

Considering above view the experimentation has been done on Leucaena leucocephala, a fast growing plant. A special emphasis has been given for those factors which limits the rate of photosynthesis i.e. environ-

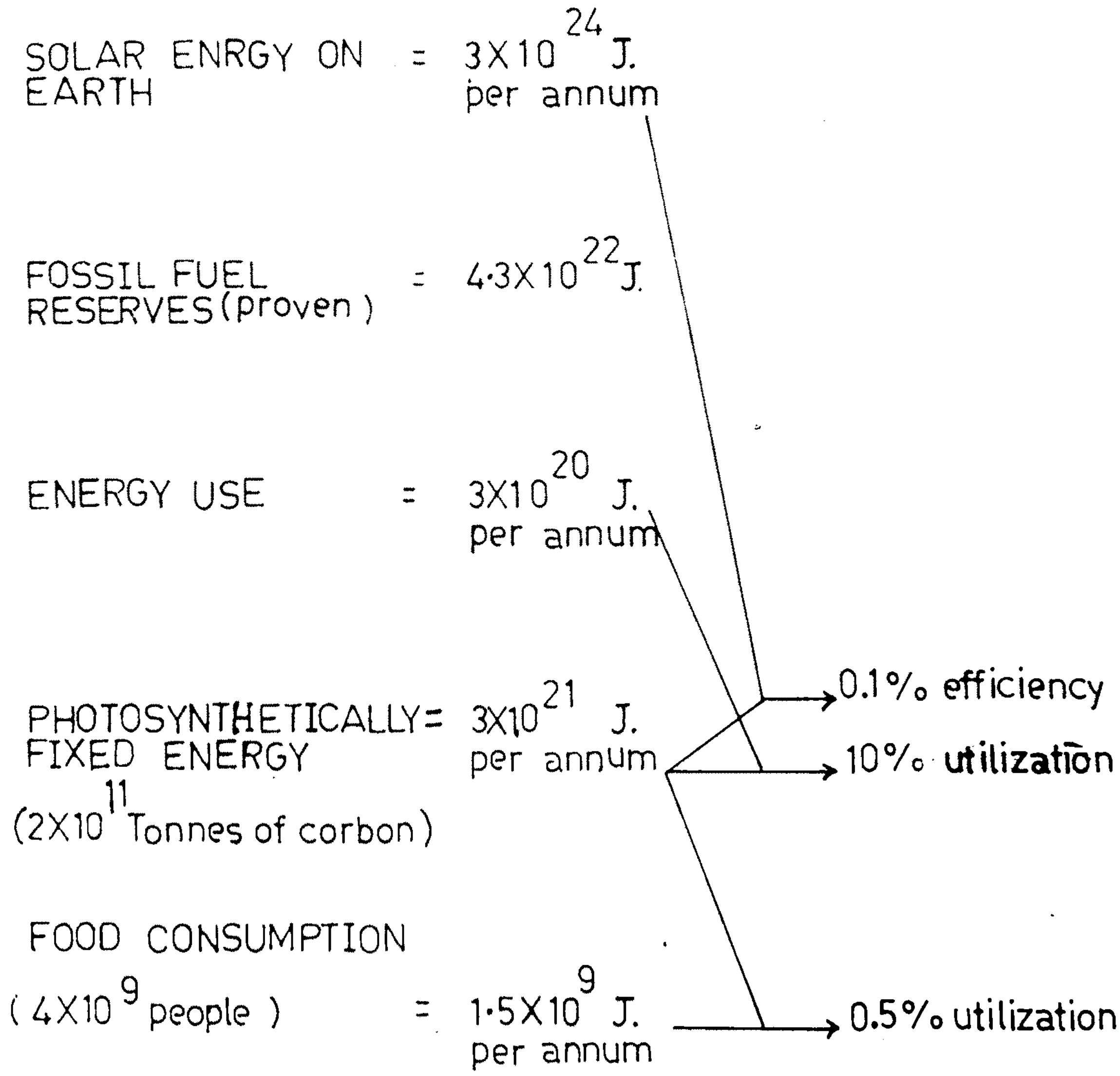

Fig. 3.2 : World energy balance and photosynthesis

mental factors like CO_2 concentration, leaf temperature, surrounding temperature, light intensity etc. The detailed study on effect of light intensity on the rate of photosynthesis is studied.

3.3 MATERIALS AND METHODS :

3.3.1 Photosynthetic Measurement by Nondistructive Measurement Technique :

The various methods have been recommended for determination of CO_2 assimilation rate (Sestak et.al. 1971). But, recently much attention has been devoted to studies on better utilization of biomass (Mislin 1982, Holl 1979, 1983). According to the measuring characteristics, these methods are grouped into three categories :

1) Chemical type methods
2) Physicochemical methods
3) Physical methods.

However, it is important to find out which method is suitable for laboratory and field study. As per the experimental construction, method (1) and (3) can be used only in the laboratory and not in field. On the other hand, physicochemical type methods (2) are suitable for laboratory as well as field purpose. In addition, we can find out photosynthetic activities of the plant without disturbing its natural activities. Hence, the method is also called as non-distructive measurement method. In this method the estimation of CO_2 partial pressure produced can be made by

a) through measurement of the pH of the solution by calorimetry (Catasky and Slarik, 1958).

b) by means of the measurement of the changes of conductivity produced in a sodium hydroxide solution absorbing CO_2 (Thomas, 1933), and

c) by means of continuous measurement of pH of diluted solution of bicarbonate (Zunker and Kreeb, 1970).

The pH value of a dilute solution of bicarbonate of alkali metal (usually $NaHCO_3$) is proportional to the partial CO_2 pressure, in air, in equilibrium with the solution

Air CO_2

$$\text{solution} \quad \underbrace{CO_2 + H_2O = H_2O_3}_{(4.12)} = \underbrace{H^+ + HCO_3^-}_{4.11} = HCO_3 + Na^+ = NaHCO_3$$

The equilibrium constant of (4.11) is given by

$$K = \frac{|H^+|\,|HCO_3|}{|H_2CO_3|}$$

As carbonic acid is in equillibrium with dissolved CO_2 (eq.4.12) the K can be written as

$$K = \frac{|H^+|\,|HCO_3|}{|CO_2|}$$

where $|CO_2|$, the molar concentration of dissolved CO_2

Then,

$$\frac{1}{|H^+|} \quad \frac{|HCO_3|}{K\,|CO_2|}, \quad \text{taking log}$$

$$-\log|H^+| = -\log K + \log\frac{|HCO_3|}{|CO_2|}$$

or $\quad pH = pK + \log|HCO_3| - \log|CO_2|$

Henderson-Hasselbalch equation

$|pH \;\&\; CO_2|$

3.3.2 Experimental Procedure :

To improve the sensitivity of the method, there requires precise temperature control, and pH measurement units.

The apparatus for measuring conductometric exchange consisted of air pump (500 r.p.m.) a photosynthetic glass chamber of volume 500 c.c and 15 cm. in length and reaction chamber of 500 c.c. by volume. The volume system made air tight and constructed so that it could be instanteniously changed from open to closed circuit and vice-versa for the precise measurement of pH of the solution, reaction chamber was directly connected to pH meter by probes. The experimental setup of the method is shown in fig. 3.3.

The fresh healthy plant organ of area 266.127 cm^2 was placed in the leaf chamber (photosynthetic chamber). After expiration of an air through open circuit the chamber was illuminated from top with a 1000 w. lamp. The light intensity at the chamber was monitored with a photo electric photometer (Lux meter).

The material used for study was Leucaena leucocephala species (age 6 months) having total leaf area 266.127 cm^2. The speciesd were brought from the experimental field i.e E.P.P. at Shivaji University, Kolhapur, fully expanded healthy green leaves were used for the experiment.

For the determination of CO_2 compensation point the leaf chamber was illuminated at constant, intensity. After about 30 minutes the system was changed to a closed circuit (dark). The CO_2 concentration in the circuit started to decrease. After each 10 minutes, air circulation was

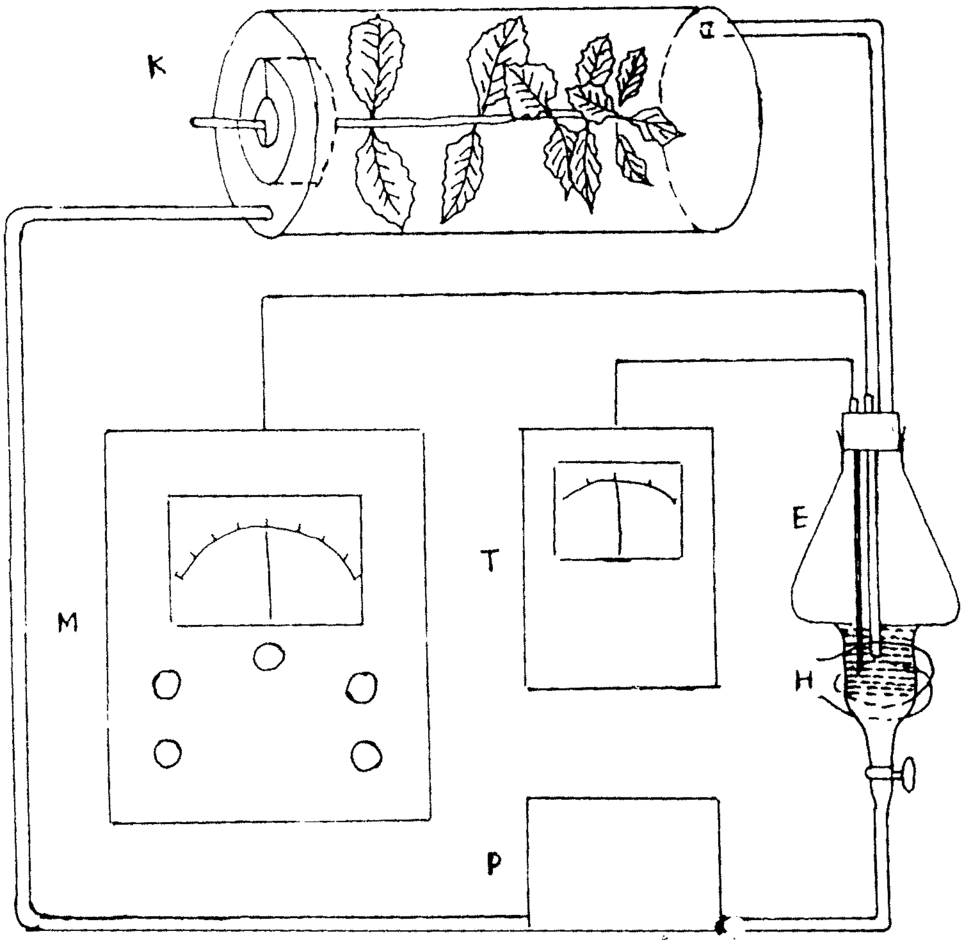

Fig. 3.3 : Experimental setup of the physico-chemical photosynthesis.

K = photosynthetic chamber made of lucite no greater than 400 to 500 cc capacity.
E-H = reaction chamber made from an Erlenmeyer flask of 50 cc to which a filter funnel with a porous glass bottom and a wrench is soldered. This chamber contains all CO_2 absorbing solution which is composed of 84.0 mg $NaHCO_3$ (=sol. 0.00 N) + 7.460 KCl (=sol.0.099N) brought to a litre with distilled water.
P = membrane pump actioned by 6-V batteries.
T = temperature sensor.
M = portable pH-meter with glass electrode.

made and change in pH were recorded. This was repeated until the decrease in CO_2 concentration in a closed circuit showed a constant reading of pH meter. Before changing the light intensity the system was changed to an open circuit and air circulation allowed to take place till meter shows constant reading. After changing light intensity the same procedure was repeated as mentioned above.

3.4 RESULTS AND DISCUSSION :

The present studies of physico-chemical activities of photosynthesis and respiration were elaborated with the help of time course of CO_2 exchange. Since net photosynthesis is limited by the available CO_2 in the closed system (air) (Calvin, 1977, 1989) it is important to optimise the volume of photosynthetic chamber and reaction chamber.

3.4.1 Photosynthesis imposed by CO_2 in *Leucaena leucocephala* :

Fig. 3.4 shows the time course of photosynthetic pH variation of bicarbonated solution, at different illumination and light intensities. In *Leucaena leucocephala* plant, Fig. 3.4 shows that, there is gradual increase in pH of the bicarbonated solution with respect to time and finally it attains the saturation value. For higher intensities, pH shows maximum values and CO_2 absorption rate is also maximum. This means in higher light intensities, *Leucaena leucocephala* plant leaves have maximum capability to absorb the photosynthetic active radiation. These radiations further used to reduce the NADP and ATP. These synthesised compounds are then used to reduce the CO_2 percentage in the leaf and this reduced percentage is filled by the atmospheric CO_2. This absorbed CO_2 in photo-

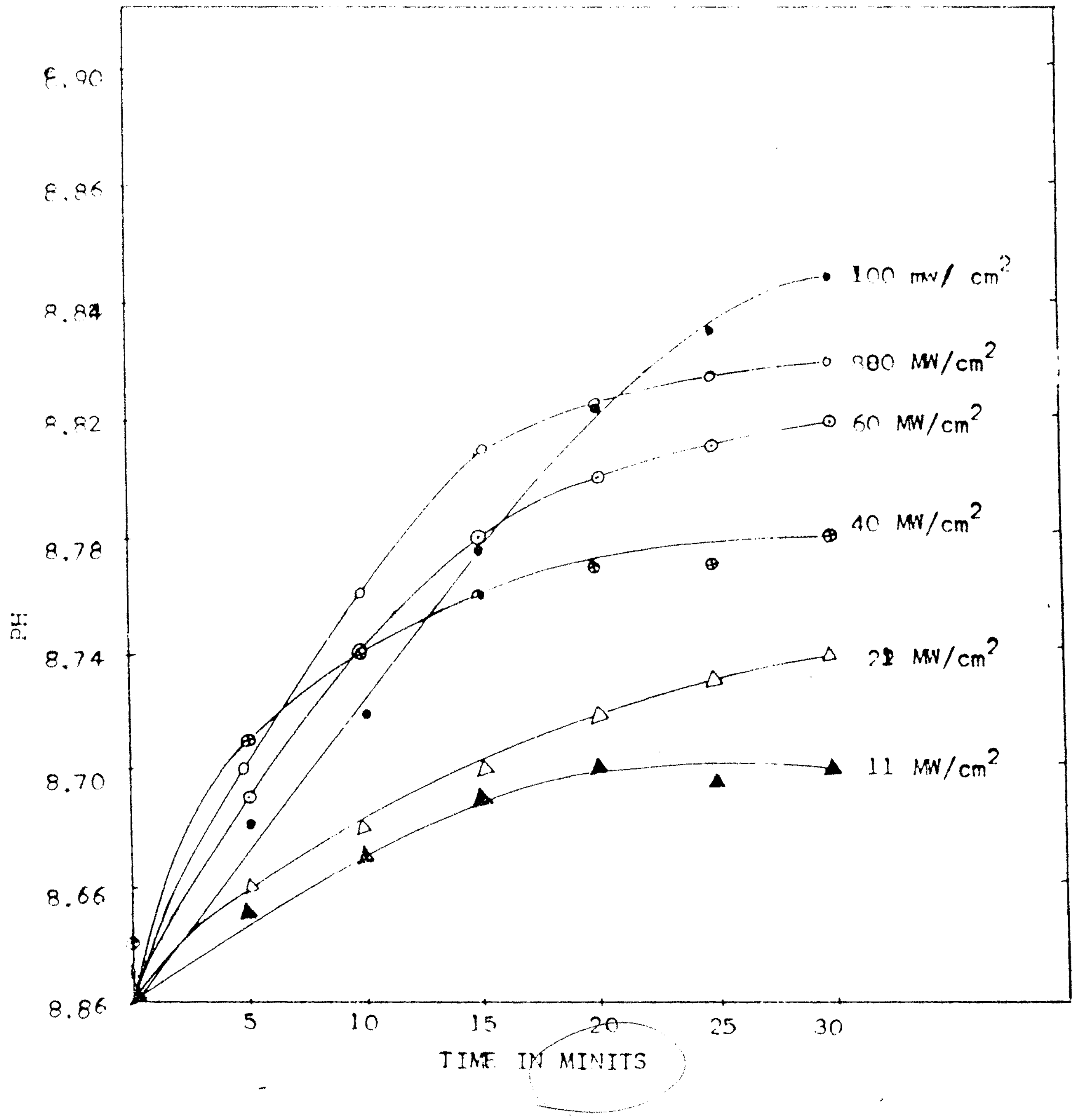

Fig. 3.4 : Time course of photosynthetic pH variation of bicarbonated solution.

synthetic chamber is counted by bicarbonated solution due to the partial pressure and pH value goes on increasing. The detailed estimation of it is shown in Fig.3.5a,bwhere it shows decreasing values of CO_2 from closed circuit as pH goes in increasing. Performance of higher light intensity on CO_2 absorption is also shown in the same figure. For higher light intensities the Leucaena leucocephala plant removes maximum CO_2 from the closed air. This result is well supported by the observations made by other workers (Tolbert 1977). The more details of the study are given in Fig. 3.6 where the time course of photosynthetic CO_2 absorption by Leucaena leucocephala is given from the figure, for lower light intensity, photosynthetic CO_2 absorption is less and vice versa. This suggests that for efficient photosynthesis in Leucaena leucocephala, higher light intensity plays an important role. But this can be restricted by available CO_2 in the close chamber (air).

3.4.2 Effect of Light Intensity on Photosynthesis :

From the Fig. 3.6 it is clear that, for lower light intensity CO_2 absorption is less and for higher intensity it is optimum. In general, it is concluded that light intensity affects on CO_2 absorption. The CO_2 absorption by plants is directly related to that of photosynthesis rate of the plant. Fig. 3.7 shows the effect of light intensity on photosynthesis rate in Leucaena leucocephala. The plot gives rate of change of CO_2 Mg/l/h (which is termed as photosynthetic rate of Leucaena leucocephala) with respect to light intensity. In initial stage, for lower light intensity (below 40 mw/cm^2), the rate of CO_2 absorption in Leucaena leucocephala increases and attains its optimum value. In final stage, for higher light

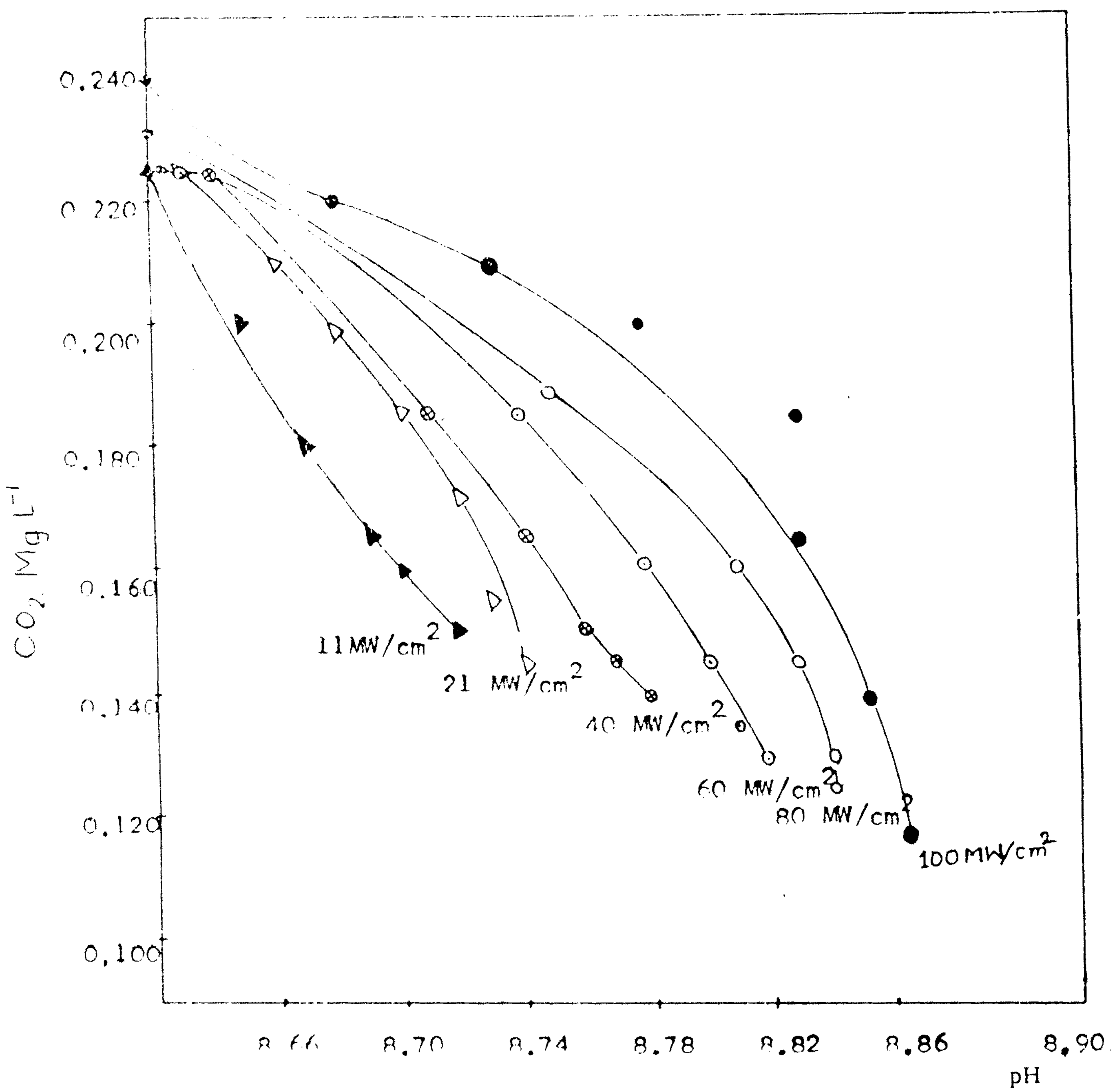

Fig. 3.5a : pH variation of bicarbonated solution during photosynthetic CO_2 uptake at different light intensity.

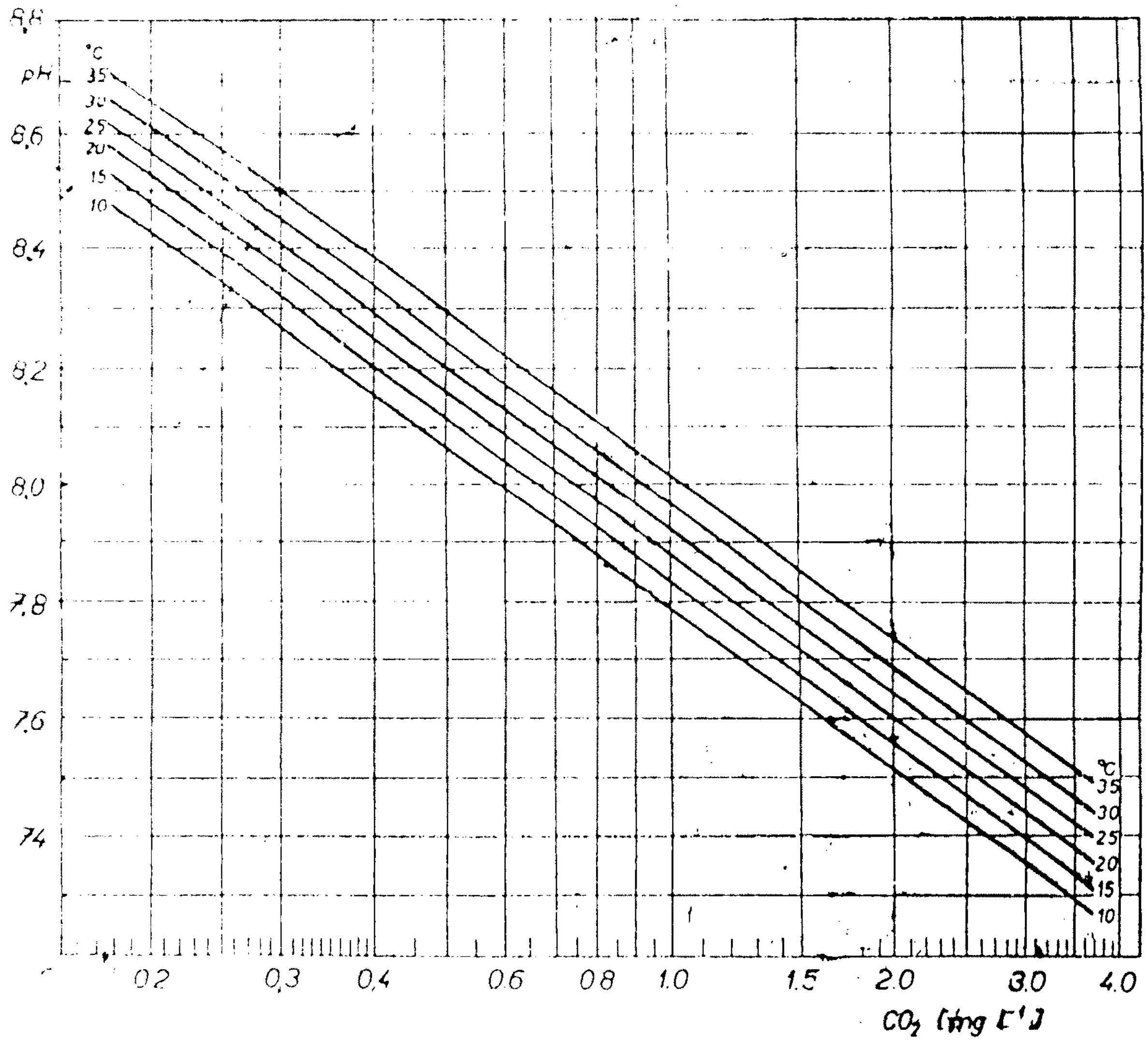

Fig. 3.5b : Ideal pH variation during CO_2 uptake at various temperatures

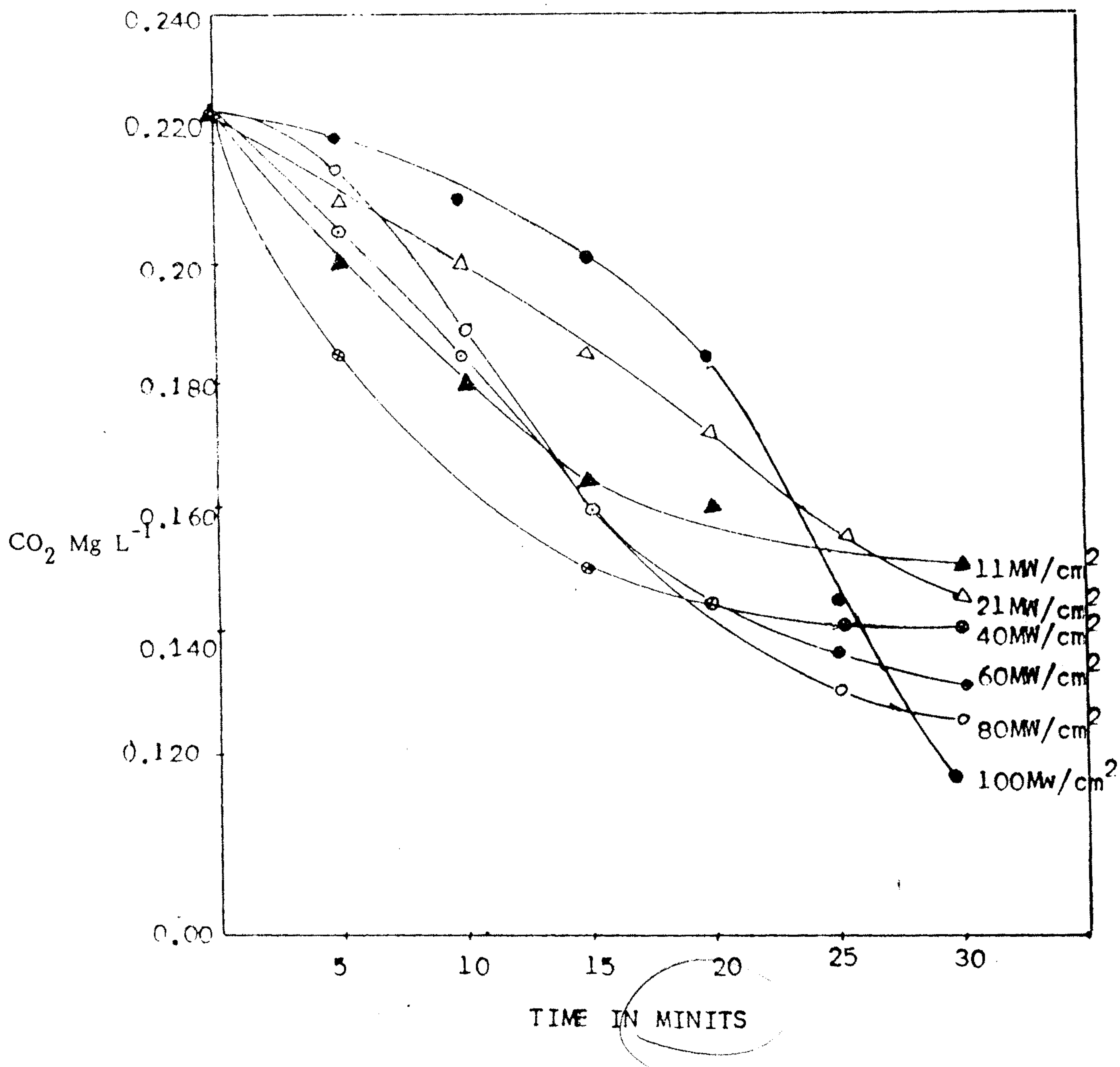

Fig. 3.6 : Time course of photosynthetic CO_2 uptake at various light intensity.

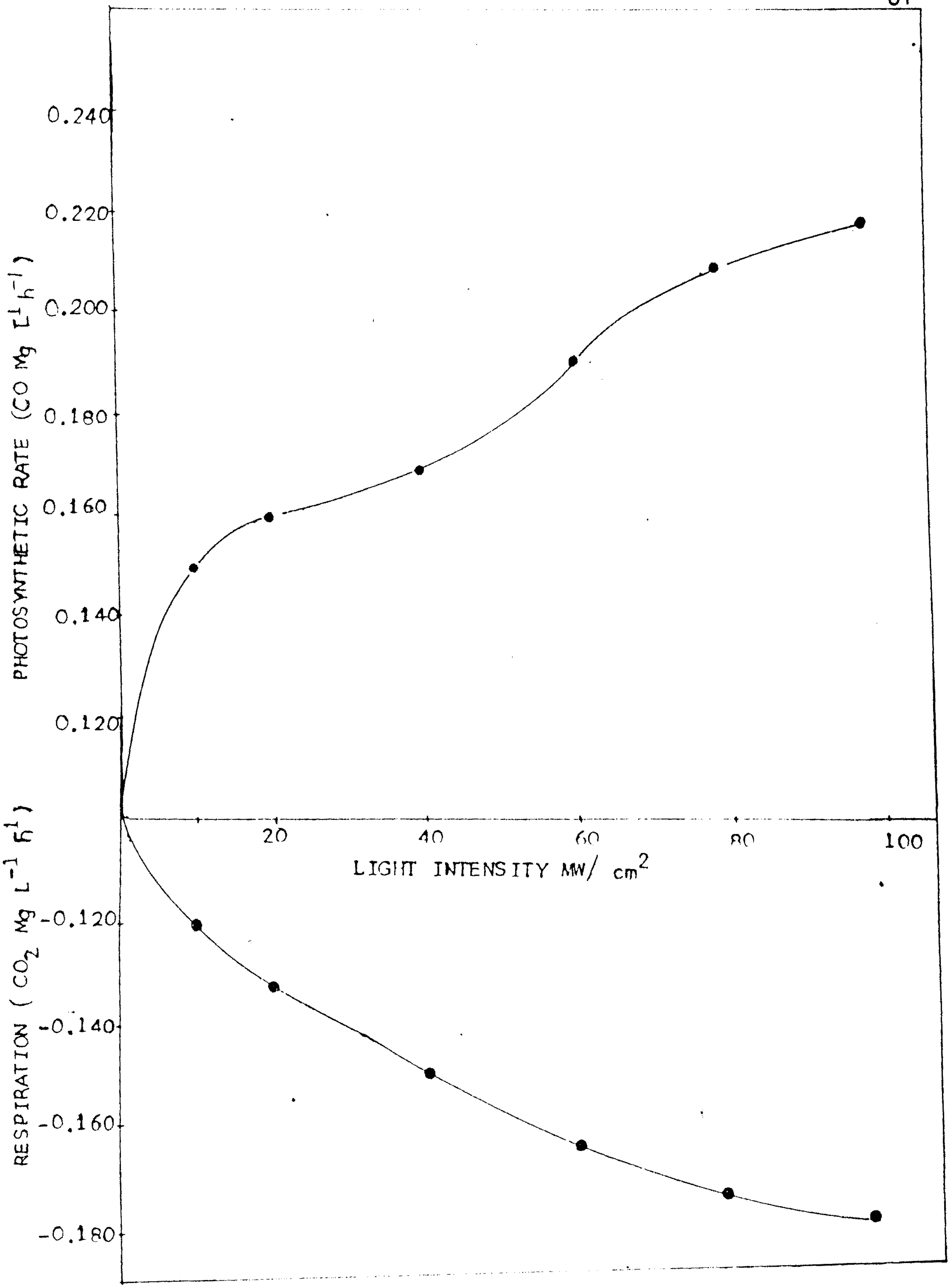

Fig. 3.7 : Photosynthetic and respiratory rate in Leucaena leucocephala

intensity (above 40 mw/cm^2) same case is observed. In both these cases the rate of CO_2 utilization by plant is less compared to the CO_2 utilization in the range 40 to 60 mw/cm^2. This is because in initial stage due to the lower intensity plant requires more time for optimum absorption of photosynthetic active radiation. In final stage due to maximum light intensity plant organ utilizing its more energy to absorb the photosynthetic active radiation more and more in short period but could not utilize all absorbed CO_2 properly. In this case, temperature goes on increasing resulting less utilization of CO_2. On the other hand at 40 mw/cm^2 the CO_2 utilization time is maximum where photosynthetic rate attends its optimum value. Hence, intensity between 20 mw/cm^2 to 60 mw/cm^2 is good for. Photosynthesis in plant at 40 mw/cm^2 it is optimum. This result is also supported by other workers (Zelawski 1967). From the photosynthetic activities of the plant and characteristic plot of photosynthetic rate is found that Leucaena leucocephala is from C_3 category type plant species.

3.4.3 Respiration Imposed by CO_2 in Leucaena leucocephala :

In the process of photosynthesis, (which mainly occurs in chloroplast) CO_2 is reduced to sugars and oxidised back to CO_2. The reduced CO_2 is used for biological growth process. The whole process involved in dark termed as 'dark respiration'. In this process synthesis of ATP (Adinosintry phosphate) occurs. Hence in biomass (plant) production, respiration process plays an important role. The studies on time course of respiratory pH variation of bicarbonated solution at various light intensity on Leucaena leucocephala is shown in Fig. 3.8. From the graph it is observed that, the

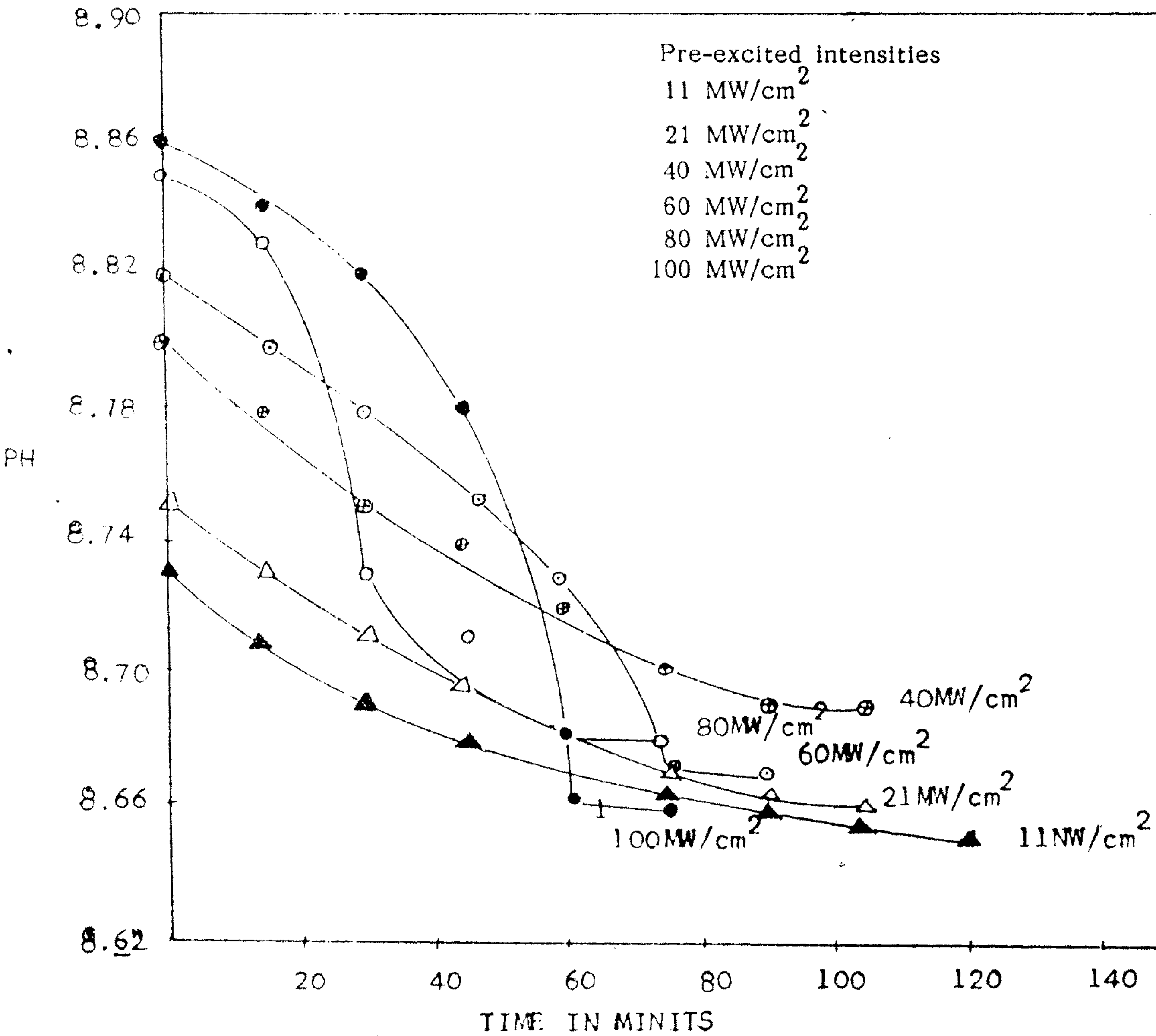

Fig. 3.8 : Time course of respiratory pH variation of bicarbonated solution at different light intensity.

collected CO_2 in the photosynthetic chamber is absorbed by bicarbonated solution (due to the partial pressure developed in the reaction chamber) and pH goes on decreasing with respective to time. This indicates that, produced CO_2 during oxidation reaction gives out to the atmosphere with that of the water. The CO_2 evolution rate depends on the respiratory time as respiratory time goes on increasing. CO_2 evolution goes on increasing at ultimately pH goes on decreasing. The pH variation during respiratory CO_2 evolution at various light intensities is shown in Fig. 3.9.

3.4.4 Effect of Light Intensity on Respiration in *Leucaena leucocephala* :

The effect of light intensity on respiratory CO_2 evolution in *Leucaena leucocephala* is shown in Fig. 3.10. From the graph it is observed that at higher light intensity (100 mw/cm^2) CO_2 evolution rate goes on increasing and for lower light intensity (100 mw/cm^2) it is less. This indicates that at higher light intensity respiratory CO_2 and water evolution rate is more. This is because, in higher light intensity temp. of the surrounding goes on increasing and to maintain the body temperature. Plant removes its maximum water as well as CO_2 with less utilization of it. From the overall observation, it is concluded that for efficient biomass production, respiratory CO_2 evolution rate should be minimum so that plant could utilize more CO_2 for biological growth process. It has also been found that respiratory activities enhances as intensity of incident light is increased from 20 mw/cm^2 to 60 mw/cm^2 and is supported by other workers also (Brix 1968, Hew 1989, Hamgren 1967, Jolitte 1968, Parkuta 1968, Tranquenna 1966, Zolawski 1967 and Zelic 1971).

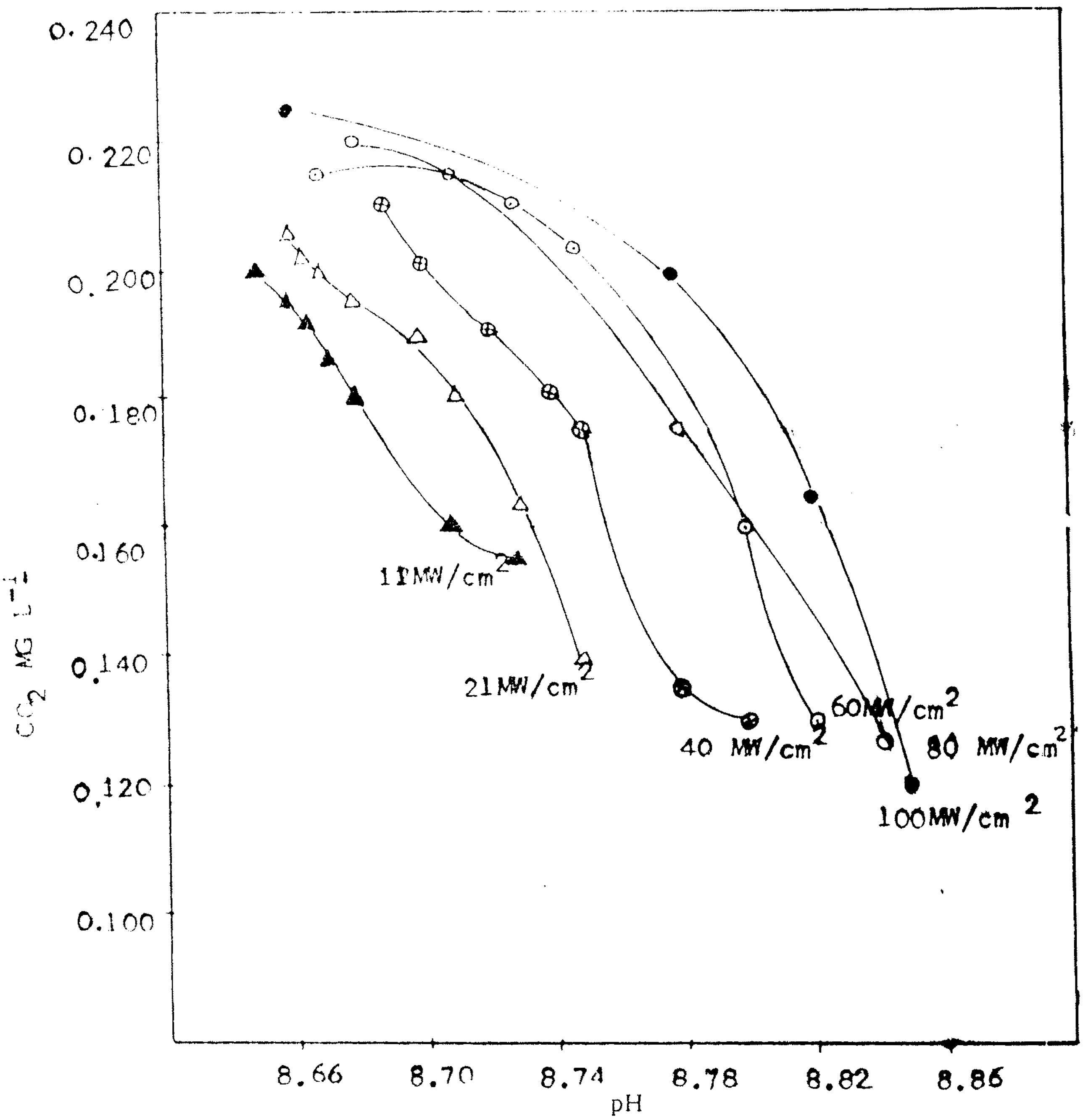

Fig. 3.9 : pH variation during respiratory CO_2 evolution at different light intensity.

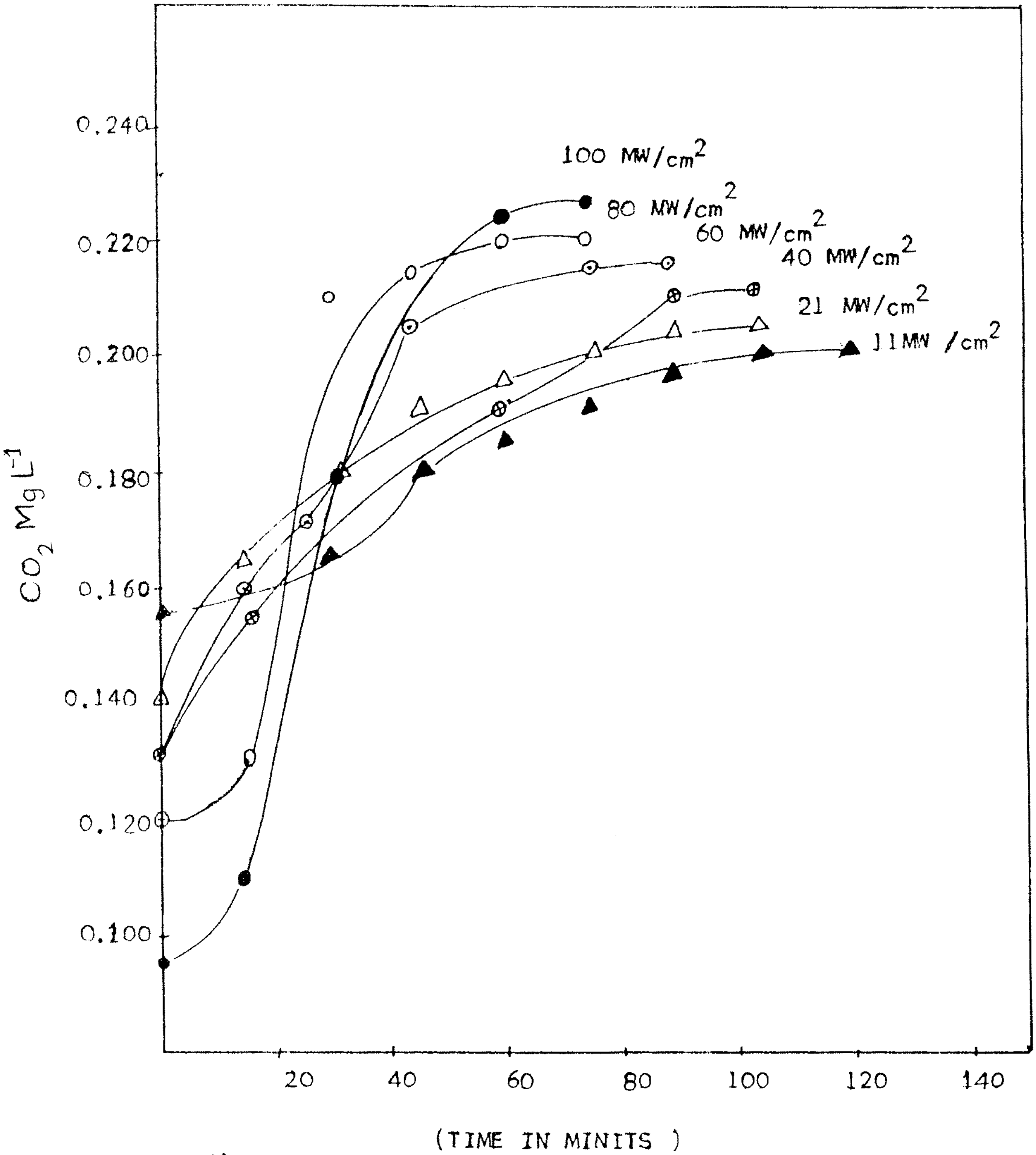

Fig. 3.10 : Time course of respiratory CO_2 evolution by <u>Leucaena leucocephala</u> at various light intensity

3.4.5 CO_2 Compensation Point in Leucaena leucocephala :

Every plant has its own capacity to utilize CO_2 for its biological growth process. In Leucaena leucocephala CO_2 utilization capacity was studied with the help of CO_2 compensating point between photosynthesis process and respiratory process. The Fig. 3.11 gives the nature of CO_2 compensation point in Leucacena leucocephala. It is observed that at higher light intensity CO_2 compensation point (CO_2 utilization capacity) is less, wh8ile at lower light intensity it is maximum. That means at lower light intensity CO_2 utilization capacity is maximum, but it requires more time to complete the process. From the graph, it is also shown that as light intensity goes on increasing CO_2 compensation point goes on decreasing. In the intensity range between 40 mw/cm^2 to 6 mw/cm^2 the optimum CO_2 utilization occures it.

The optimum CO_2 compensation point observes at 40 to 60 mw/cm^2 The nature of the plot also shows that CO_2 compensation point goes on decreasing with respective to light intensity and reaches its steady state value. This result of CO_2 compensation point nature in Leucaena leucocephala plants therefore, agree with the generally accepted concept of C_3 plant species (Plaiwal et.al. and Guanam et.al. 1986).

3.5 CONCLUSION :

The conclusions obtained from overall study of photosynthesis in Leucaena leucocephala are discussed in below lines.

1) The method used for photosynthesis study i.e. physico-chemical method is best method for laboratory as well as field study.

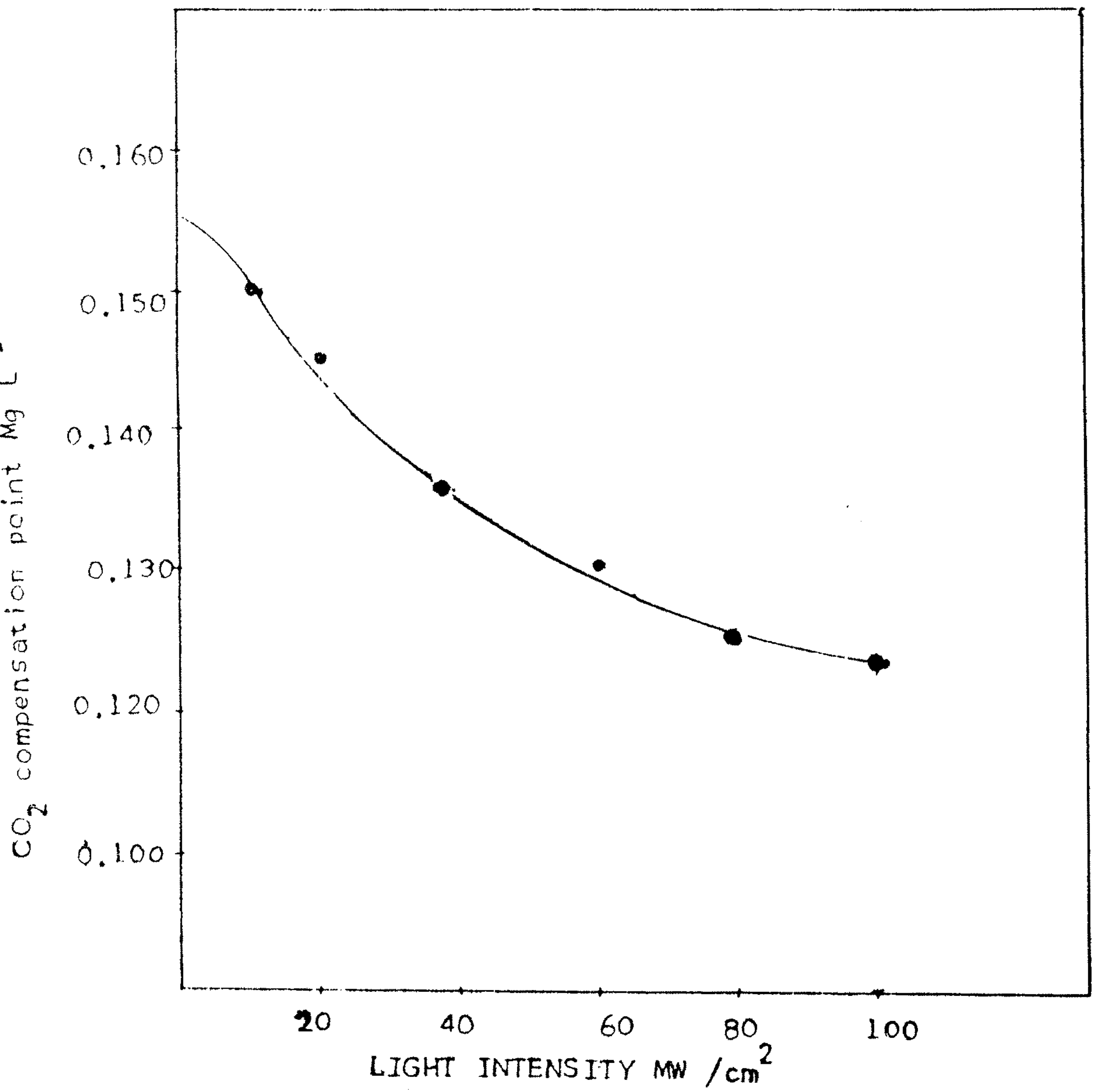

Fig. 3.11 : Effect of light intensity on CO_2 compensation point

2) For accurate measurement of photosynthetic rate precise measurement of pH requires.

3) The optimization of various parameters like size of photosynthetic chamber, reaction chamber and temperature of both these chambers are very essential.

4) The time course of photosynthetic pH variation of bicarbonated solution at different illumination of light intensities on Leucaena leucocephala shows that, there is gradual increase in pH with respect to time which conclude during the light process. Plant absorbs more CO_2 from its surrounding.

5) The effect of light intensity on photosynthesis rate indicates that at 40 mw/cm^2 light intensity photosynthetic rate is optimum and plant utilizing more CO_2 from the atmosphere.

6) In the process of respiration in Leucaena leucocephala, it is concluded that, for biological growth process respiration process should be optimum, so that plant could not remove maximum CO_2 to the atmosphere.

7) At various light intensities, the respiratory process has different reaction rate and CO_2 evolution rate. It is concluded that at light intensity 40 mw/cm^2. The respiratory process is optimum and CO_2 evolution rate is also optimum which is suitable intensity for biological growth process.

8) The utilization of CO_2 for biological growth process is also important factor for the study of photosynthesis and respiration in Leucaena leucocephala.

9) The compensation point of Leucaena leucocephala for various light intensisites shows that Leucaena leucocephala is the plant species from the generally accepted concept of C_3 plant species.

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