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Research Article

Assessment of Water Status in Medicinal Plants Using Nuclear Technique

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ABSTRACT

Medicinal plants have great importance for human health and are widely used in the treatment of many diseases like fever, diabetes, cough, stomach, cancer, joint pains and jaundice, etc. These plants have been of great demand in the area of cosmetic and pharmaceutical industries.The quality of the medicinal plants depends upon the water content as water stress generally reduces fruit size, fruit pulp and growth mainly in young trees. Hence, it is important to management the water status in the medicinal plants. The studies of beta attenuation coefficient have helped a lot in solving variety of problems in agricultural, physical and medical sciences. Therefore, the present study was carried out for determination of water content in the plant leaves of Tulsi (*Ocimum sanctum*) based upon their characteristics of attenuation to the beta particles. The Geiger Muller Counter was used as detector for the beta attenuation measurement of leaf samples. The intensities of transmittance and mass attenuation coefficients, through leaves of different thickness were measured. These parameters vary as the amount of water content in selected leaves get changed. The mass attenuation coefficient of Tulsi leaves is found to be decreased with decrease in water content of leaves. These results of present study indicated that the nuclear technique used is non-destructive, easy to handle and efficient method for evaluation of water status of these plants to their optimum growth.

Key words: Medicinal plants, GM counter, Beta particle, Attenuation coefficient, Water status

Introduction

Plants play very important role in the global circulation of water and conversely, water is necessary for the germination of seeds, growth of plant, and transpiration. Shortage of water will cause wilting of leaves. Water helps in transportation of important nutrients through the plant (Sood, 2016). The stress of water alters the balance between the production of reactive oxygen species (ROS) and the antioxidant defence, causing the accumulation of ROS that leads to oxidative stress to proteins, membrane lipids and other cellular components. Drought stress also has a negative influence over photosynthesis due to interference in stomatal functioning and damaging the photosynthetic apparatus severely *via* ROS production. Hence water stressed plants exhibit disturbed metabolism of carbon, nitrogen and oxygen particularly (Moinuddin *et al.,* 2012).

Water status of plant can be estimated by the features of tissues such as root, stem and leaf or the whole canopy. In comparison to the other tissues, leaf analysis is widely used tool for evaluating nutrients and water status of plant, which are used for guiding irrigation and fertilization of plant. There are various methods to get water content of leaves (Sood and Singh,

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2016). Turner (1988) used pressure chamber technique to measure leaf water potential by studying the total water potential and pressure volume relations of leaves and roots. Hunt and Rock (1989) estimated plant water stress by remote sensing, using indices of Near Infrared (NIR, 0.7-1.3 pm) and Middle Infrared (MIR, 1.3-2.5 um) wavelengths. Afzal *et al.* (2010) measured the water content of leaf by the variation of capacitance measured using designed capacitive sensors. They estimated the leaf water content by monitoring its capacitance for five agronomic crops. Generally, the internal water status of plant is estimated by the Diffusion Pressure Deficit method (Slatyer, 1958), the Relative Turgidity method (Weatherley, 1950; Barrs and Weatherley, 1962) and the β gauging method (Obrigewitsch *et al*. 1975). But first two methods need a controlled environment and are time consuming, hence can't be used for monitoring of plant water status. The β attenuation technique is being widely used to monitor continuously the water contents of plant leaves (Mederski, 1961; Nakayama and Ehrler, 1964; Rolston and Horton, 1968; Mederski and Alles, 1968).

Attenuation is reduction of intensity of radiation due to absorption or scattering, as they pass through the matter (Pattanashetti and Galagali, 2016). The radiation interacts with matter at the fundamental levels of atoms or their more elementary constituents such as electrons and the nuclei. Beta particle attenuation can be used to extract information about composition of matter such as thickness, water content etc. (Kirandeep *et al*., 2015). The attenuation studies in matter have helped a lot in solving variety of problems in physical sciences, bio-sciences, agricultural sciences and medical physics. Jarvis and Slatyer (1966), Whiteman and Wilson (1963), Jones (1973) and Obrigewitsch *et al*. (1975) formed a relation between count rate of β particles and relative water content (RWC) of the leaves. But this method requires calibration with fully turgid leaf in addition to fresh and dry leaves (Nielson and Cassel, 1984). However, when leaves are kept in distilled water under suitable light and temperature conditions (Obrigewitsch *et al*., 1975) for about 3-4 hours, there is loss of organic matter into water and hence, the beta gauging technique is inappropriate to use. The present technique will overcome the limitations of the β gauging technique.

In the present study, the absolute water content of Tulsi leaves is estimated by monitoring transmission of β particles through fresh and completely dry leaves. The relation between the measured mass attenuation coefficients and relative intensities for fresh and dry leaves is established and absolute water content in the leaf is determined. The temperature dependence of water status of Tulsi leaves is monitored at various temperatures.

The beta radiations are high energy electrons emitted from the nucleus. These interact with matter in various ways and lose their energy. Some of these undergo inelastic collision with atomic electrons, bremsstrahlung *i.e.* deflection of incident beam through field of nucleus, and elastic collision of incident electrons with atomic electrons (Singru, 1974). Due to which the incident beam loses its energy and gets attenuated. Attenuation of radiation depends upon geometry and composition of the material. Major constituent in a plant leaf is water. So attenuation of radiation is mainly caused by water. The attenuation of beta radiation through a plant leaf depends upon mass per unit area of the leaf.

The intensity of transmitted beta radiation through a fresh plant leaf, is given by

$$I_f = I_o \exp^{-\mu_f t_f} \qquad \ldots(1)$$

Where I_o is the intensity of the incident beta radiation, μ_f and t_f are the mass attenuation coefficient and thickness respectively of the fresh plant leaf (organic matter and water).The mass attenuation coefficient can be determined by plotting graph of relative intensity versus thickness of leaf. The above equation can be rewritten as below

$$t_f = \frac{1}{\mu_f} \ln\left(\frac{I_o}{I_f}\right) \qquad \ldots(2)$$

Where t_f, I_f and μ_f are the mass per unit area, intensity and mass attenuation coefficient respectively of fresh leaf.

Similarly for a dry leaf

$$t_d = \frac{1}{\mu_d} \ln\left(\frac{I_o}{I_d}\right) \quad \ldots(3)$$

Where t_d, I_d and μ_d are the mass per unit area, intensity and mass attenuation coefficient respectively of dry leaf (organic matter)

Since leaves contain organic matter and water, so mass of water per unit area can be written as

$$t_w = t_f - t_d \quad \ldots(4)$$

$$t_w = \frac{1}{\mu_d} \ln\left[\left(\frac{I_o}{I_f}\right)^n \times \left(\frac{I_d}{I_o}\right)\right] \quad \ldots(5)$$

where $n = \mu_d/\mu_f$, the ratio of mass attenuation coefficients of dry leaves to that of fresh leaves. In this way, the water content of plant leaves can be determined by using all the variables.

Materials and Methods

The experimental set up consists of :

a) Radioactive source: Co-60 is used as source of beta radiation.

b) Absorber holder: Tulsi leaves are kept in between aluminium sheets, of size 6.5×6.5cm, with matching holes of size 1.8cm.

c) Detector: The Gieger Muller counter is used for measurement of transmitted intensity through absorber.

The experimental arrangement is shown in figure 1.

Tulsi leaves of size 2×2cm were cut from whole leaf sample and kept in between the absorber holders (placed in the 1st shelf of GM tube). The source was kept on the second shelf of the GM tube. The geometry of source, absorber and the detector were centrally aligned. Voltage across of GM tube was kept 850V i.e. in the operating region of counter. The transmitted intensity was measured by GM counter and the observation time was chosen 60 seconds. Similarly, readings for various thicknesses of leaves were taken by placing more leaves in between the aluminium sheets. Mass per unit area was determined by using a sensitive weighing balance. Two methods were used to dry the Tulsi leaves. In first method, leaves were kept to dry for 6 days at room temperature and measurements were done same as for fresh leaves (Kirandeep *et al*. 2015; Pattanashetti and Galagali, 2016). In second method, Tulsi leaves were kept in oven to dry for one hour at temperatures 30°C, 40°C and 50°C and then measurements of transmitted intensity and mass per unit area of these leaves were determined similar as in the case of fresh leaves.

Results and Discussion

The data for Tulsi leaf thickness and number of counts has been shown in Table 1 for fresh

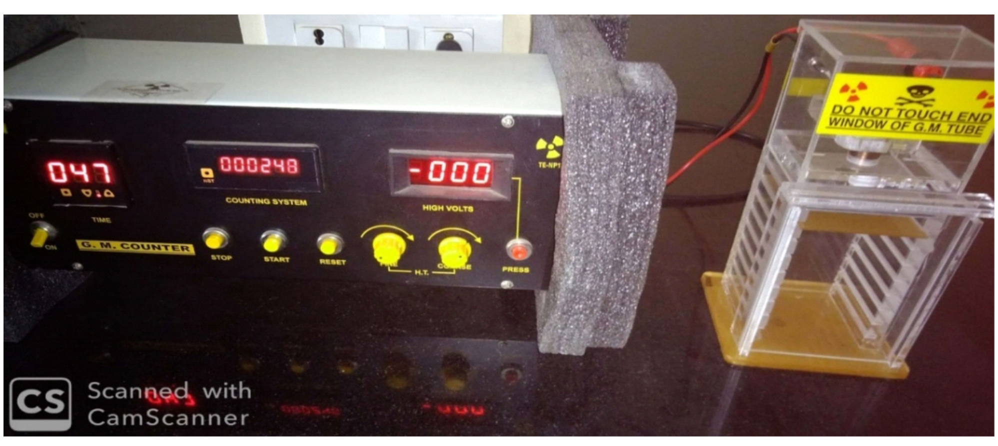

Fig. 1. Experimental setup

Table 1. Properties of fresh and dry Tulsi leaves obtained from Co-60 source

S. No.	Thickness (mg/cm^2) of fresh leaves	Number of counts per sec. (N) for fresh leaves	Thickness (mg/cm^2) of dry leaves	Number of counts per sec. (N) for dry leaves
1.	11.5	28.88	4.3	43.62
2.	24.8	20.17	7.6	39.97
3.	36.5	18.19	13.8	29.88
4.	49.9	16.80	16.3	23.46
5.	63.7	14.17	21.1	18.44
6.	72.3	13.21	24.6	16.80
7.	84.4	11.92	29.3	13.65
8.	118.7	11.25	34.3	12.78

(a) Fresh leaf (b) Dry leaf

Fig. 2. Fresh and dry Tulsi leaf

and dry leaves. The thickness of fresh leaf contains organic matter and water content but that of dry leaf contains organic matter only as shown in figure 2 respectively.

The number of counts versus thickness of fresh and dry Tulsi leaves are shown in fig. 3a&b, respectively. It can be seen that the number of counts decreases exponentially with increasing the thickness of leaves which is in line with the studies reported by Kirandeep *et al*. (2015) and Pattanashetti and Galagali (2016).

Table 2 contains the data of Tulsi leaves kept at temperature 30°C in oven for one hour. The column 4 contains the logarithm of relative number of counts where N_o is the number of counts in the absence of leaves. Table 3 and 4 contains the data of leaves at temperatures 40°C and 50°C, respectively.

The thickness (mg/cm^2) versus logarithm of relative number of counts for Tulsi leaves at temperature 30°C, 40°C and 50°C are shown in fig. 4 a,b & c, respectively. The mass attenuation coefficient is given by the slope of graph for each case. These figures show that the mass attenuation coefficients of Tulsi leaves decrease with temperature (Kirandeep *et al*., 2015; Pattanashetti and Galagali, 2016; Sood, 2016). This is due to the fact that more water gets evaporated at high temperature and there is less water content in the

Table 2. Tulsi leaf kept at temperature 30°C for one hour

S.No.	Thickness (mg/cm^2)	Number of counts per sec. (N)	ln (No/N)
1.	11.3	24.95	1.035
2.	24.8	22.29	1.148
3.	40.0	17.35	1.398
4.	53.7	14.51	1.577
5.	66.2	13.98	1.615
6.	79.6	13.42	1.656
7.	93.8	11.60	1.801
8.	107.1	11.37	1.821

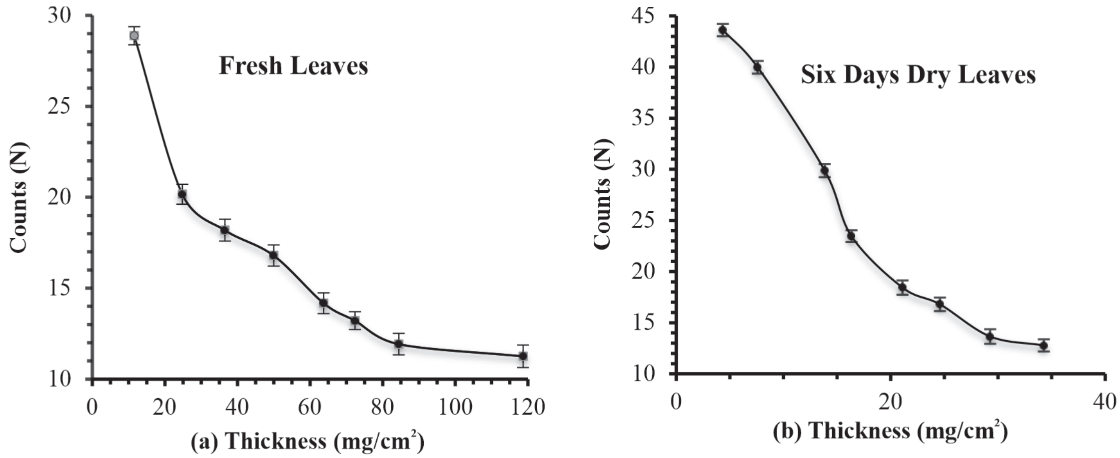

Fig. 3. Plot of number of counts Vs thickness of fresh leaf and dry leaf

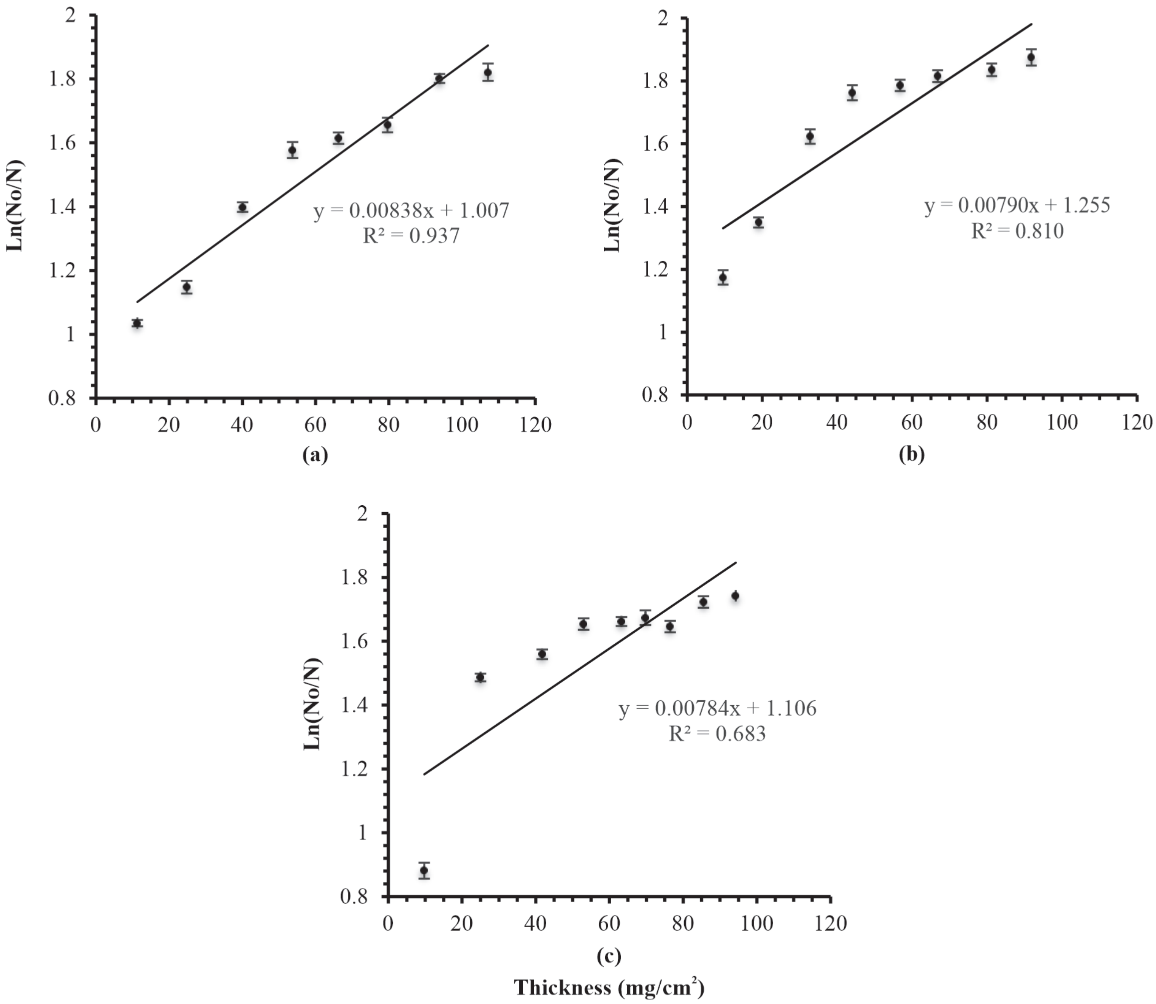

Fig. 4. a,b and c: Relative transmission intensity Vs thickness of Tulsi leaves at temperature 30°C, 40°C and 50°C, respectively.

Table 3. Tulsi leaf kept at temperature 40°C for one hour

S.No.	Thickness (mg/cm^2)	Number of counts per sec. (N)	ln (No/N)
1.	9.5	21.71	1.174
2.	19.0	18.23	1.350
3.	32.8	13.86	1.622
4.	44.0	12.06	1.762
5.	56.8	11.78	1.786
6.	66.8	11.44	1.816
7.	81.2	11.21	1.835
8.	91.8	10.77	1.875

Table 4. Tulsi leaf kept at temperature 50°C for one hour

S.No.	Thickness (mg/cm^2)	Number of counts per sec. (N)	ln (No/N)
1.	9.7	29.10	0.881
2.	25.0	15.59	1.487
3.	41.7	14.78	1.559
4.	53.0	13.44	1.654
5.	63.5	13.34	1.662
6.	69.7	13.18	1.674
7.	76.5	13.55	1.646
8.	85.5	12.54	1.723
9.	94.2	12.29	1.743

leaf. So this is a successful tool to monitor the water status of plant continuously.

Conclusions

The study showed that the mass absorption coefficients of fresh leaves was greater than that of the dry leaves as dry leaves had less water content as compared to the fresh leaves. The mass attenuation coefficient of leaves decreased with increase in the temperature being used for drying the leaves. The results indicated that lesser the water content in the leaves, more transmission of beta radiation. This knowledge of water status of plants might help to determine the plant health and quality of crop yield. The nuclear technique could be used to manage the irrigation scheduling and also for increasing the productivity of summer vegetables especially grown in areas of arid and semi-arid regions.

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