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A COMPARISON OF DIMETHYL SULPHOXIDE (DMSO) AND ACETONE EXTRACTS FOR THE DETERMINATION OF CHLOROPHYLL IN *HEVEA* LEAF TISSUE

Many plant physiological, ecological and horticultural studies require comparative analysis of leaf chlorophyll density. Water soluble solvents such as methanol, ethanol, acetone, pyridine and acetone plus ethyl acetate are in use for the extraction of chlorophylls (Strain and Svec, 1966). The conventional chlorophyll extraction method (Arnon, 1949) involves grinding the plant tissues in 80 per cent acetone with subsequent centrifugation to remove solid plant materials. This method is slow and tedious. Moreover, extracts are unstable and require immediate spectroscopic analysis. Shoaf and Lium (1976) and Hiscox and Israelstam (1979) have shown that dimethyl sulphoxide (DMSO) is superior to acetone in the extraction of chlorophylls in a wide range of algal as well as angiosperm and gymnosperm materials. They have shown that spectroscopic analysis need not necessarily be immediate since DMSO extracts are stable.

Studies on screening of *Hevea* clones for characters such as photosynthetic efficiency invariably involve estimation of chlorophylls and quite often a large number of samples are to be analysed. In laboratories with limited manpower and facilities a rapid extraction procedure in which the extractant is fairly stable, so that spectrophotometric analysis could be carried out over an extended period, is necessary. The success of DMSO method has been established in other plant materials and hence it was attempted to check its fitness in *Hevea* foliage. The study was aimed at standardisation of the DMSO extraction method for *Hevea* foliage and its comparison with acetone extraction method.

Mature leaves from eighteen trees of *Hevea brasiliensis* (clone RRIM 600), at two and a half years growth, were collected. Fresh leaf discs taken from each sample were transferred to a beaker containing 7 ml of DMSO (E. Merck Limited). The chlorophyll was extracted in the fluid without maceration by keeping on a water bath at 65–70°C for varying times viz., 15, 30 and 60 min. The extract was made up to 10 ml with DMSO and the OD was read at 645 and 663 nm in a Shimadzu UV 160 A spectrophotometer against DMSO blank. Chlorophyll, extracted in 80 per cent acetone (Arnon, 1949), served for comparison.

The samples from DMSO extracts (taken from 30 min incubation period) as well as the acetone extracts were transferred to vials, sealed and stored between 0 – 4°C. The OD values at 645 and 663 nm were read after 24, 48,72 and 96 h for determination of chlorophyll contents.

The mean values for chlorophyll content in DMSO extracts and acetone extracts are given in Table 1. There was no significant difference between acetone extraction and DMSO extraction for 15 min as evidenced by a non-significant paired 't' test value denoting that extraction for 15 min in DMSO compares well with acetone extraction. This, however is a deviation from the observations reported for some angiosperm and gymnosperm materials by

Hiscox and Israelstam (1979). According to them an extraction of 30 min with DMSO was required to obtain comparable chlorophyll values in the materials tested by them.

In the present study extraction with DMSO with 30 and 60 min. duration also was carried out. The chlorophyll estimated in extracts with both the above durations significantly differed with the extraction by acetone method for 15 min. Incubation with DMSO for 30 and 60 min was found to extract 2.87 and 2.88 mg chlorophyll per g fresh leaf, respectively as against 2.78 mg per g by the acetone extraction method and 2.79 mg per g by DMSO extraction for 15 min. The chlorophyll content by DMSO extraction with different durations revealed that extraction for 30 and 60 min. gave significantly higher content of chlorophyll compared to extraction for 15 min ($P = 0.01$) But there was no significant difference between incubation of 30 and 60 min on extraction of chlorophylls. Incubation for 30 min. with DMSO gave better extraction of chlorophyll and this could be considered optimum for the determination of chlorophyll in *Hevea* foliage.

Table 1. **Chlorophyll concentration in *Hevea* leaf tissue extracted by grinding in acetone and incubated without grinding in DMSO**

Extractants	Incubation time in DMSO (min)	Mean chlorophyll content (mg/g fw ± SE)	Paired t value between acetone and DMSO
Acetone	—	2.78 ± 0.167	—
DMSO	15	2.79 ± 0.079	0.40 NS
DMSO	30	2.87 ± 0.111	3.24 **
DMSO	60	2.88 ± 0.074	3.25 **

DMSO 15 min. V_s DMSO 30 min. t = 2.96 **
DMSO 15 min. V_s DMSO 60 min. t = 3.61 **
DMSO 30 min. V_s DMSO 60 min. t = 0.47

Table 2. **Comparison of stability of DMSO and acetone extracts : influence of storing on chlorophyll concentration in *Hevea* foliage**

Time after extraction (days)	Chlorophyll concentration (mg/g fw ± SE)		t value between 0 and 4 days	
	Acetone	DMSO	Acetone	DMSO
0	2.78 ± 0.17	2.87 ± 0.11	—	—
1	2.72 ± 0.15	2.87 ± 0.14	0.71 NS	0.04 NS
2	2.51 ± 0.19	2.84 ± 0.14	2.81 *	0.87 NS
3	2.44 ± 0.16	2.83 ± 0.14	3.86 **	1.03 NS
4	2.23 ± 0.15	2.83 ± 0.14	5.98 **	0.99 NS

* Significant at P = 0.05
** Significant at P = 0.01
NS Not Significant

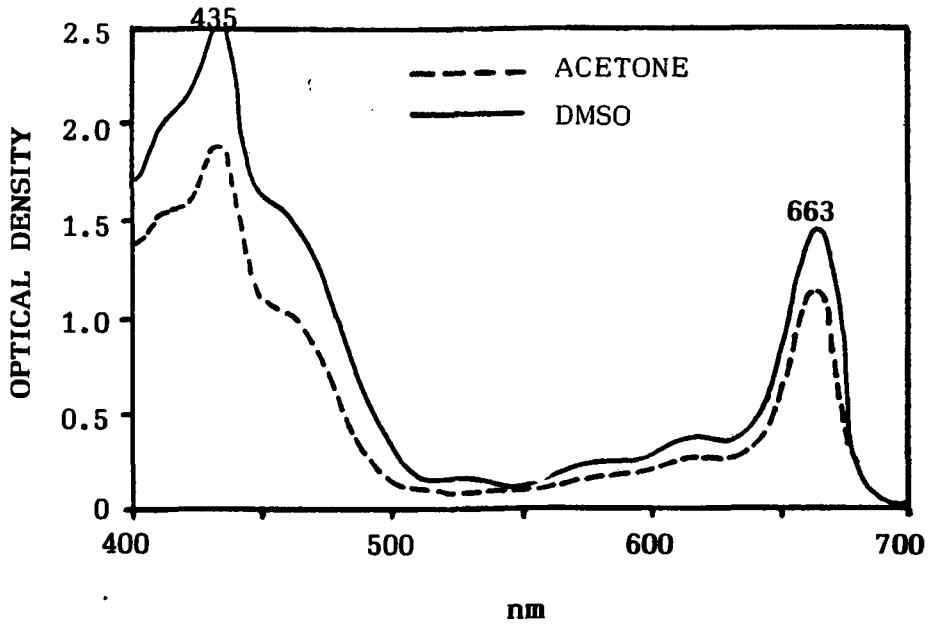

Fig. 1. Absorption spectra for chlorophyll with DMSO and acetone extraction

The chlorophyll contents decreased significantly with time in the acetone extracts but there was no significant decrease in DMSO extracts (Table 2). The per cent reduction in chlorophyll content was high (20.06 per cent on fourth day)in acetone extraction whereas, in DMSO the reduction was very less (1.56 per cent on fourth day) and remained constant afterwards. The reduction in chlorophyll content in the acetone extracts was significant with progress of time upto fourth day while there was no significant difference in DMSO extracts for the same duration. There was, however, no significant reduction for 24 h in acetone extracts.

A comparison was made using spectrophotometry (Fig. 1) between the absorption spectra (400 – 700 nm) for chlorophyll extracts prepared with DMSO and acetone. It was observed that the absorption peaks occur at the same wave lengths for both. The slightly higher OD values for the DMSO extract indicates that DMSO is more efficient in complete extraction of chlorophyll. This is in conformity with the findings of Hiscox and Israelstam (1979). Considering the stability, rapidity and also a higher extraction, the DMSO extraction could be a better method for the determination of chlorophyll in *Hevea* leaf tissue compared to the acetone extraction procedure.

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