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Extraction and estimation of chlorophyll content of seed treated lentil crop using DMSO and acetone

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Abstract
Chlorophyll is content responsible for green pigment and structurally similar to porphyrin pigments such as heme having same metabolic pathway. In present study Chlorophyll content were extracted from lentil leaves treated with bio control agent and fungicide by using dimethyl sulfoxide (DMSO) and acetone. The prepared samples were exposed to a range of light having different wavelength and analyzed using a spectrophotometer. Concentration of chlorophyll a and b was calculated using Arnon method. Chlorophyll content was higher in leaves treated with bio control agent and fungicide over control.

Keywords: chlorophyll "a", chlorophyll "b", carotenoid, lentil crop, UV-visible, acetone, spectroscopy, DMSO

Introduction
Chlorophyll is a pigment responsible for green colour, consists of tetrapyrrole ring with a central magnesium ion and having long chain of hydrophobic phytol in its structure. It is found in plants and algae (Aminot, 2000) [1]. It is of two type chl a and b, present terrestrial plants and green algae. The difference between these two chlorophylls is presence of methyl in chlorophyll a which is replaced by a formyl group in chlorophyll b. Chl a to chl b ratio is approximately 3:1 in higher plant. Chlorophyll absorbs light of different wavelength mainly the red (650 – 700 nm) and the blue - violet (400 – 500 nm) regions of the visible spectrum. Green light (~550 nm) is not absorbed but reflected giving green colour. Chlorophyll a possesses a green-blue colour, and chlorophyll b possesses a green-yellow color (Arnon, 1849). The amount of solar radiation absorbed by a leaf is largely dependent on the foliar concentrations of its photosynthetic pigments. Therefore, low Chl concentration can limit photosynthetic potential and primary production (Da Matta *et al*. 2008) [6].

In contrast to chlorophyll a and b are several non-chlorophyll accessory pigments such as carotenoids. It also absorbs light and transfer the energy to an alternate photosystem. Carotenoids also serve as antioxidants by dissipating excess light energy. Most of the chlorophyll and non-chlorophyll pigments have different spectra for one of two reasons.

Materials and methods
Collection of plants
The lentil leaves from the middle of plant is collected.

Extraction of chlorophyll and carotenoid by using Acetone
(Arnon, 1949) -0.1 g of finely cut fresh leaves were taken and ground with 10ml of 80% acetone. It was then centrifuged at 5000 –10000 rpm for 5mins. The supernatant was transferred and the procedure was repeated till the residue becomes colourless. The absorbance of the solution was red at 480, 645nm and 663nm against the solvent (acetone) blank.

Estimation of chlorophyll content
The concentrations of chlorophyll a, chlorophyll b and total chlorophyll and carotenoid were calculated using the following equation:
Total Chlorophyll: 20.2(A645) + 8.02(A663)
Chlorophyll a: 12.7(A663) – 2.69(A645)
Chlorophyll b: 22.9(A645) – 4.68(A663)
Carotenoid: [A480 +(0.114(A663)-(0.638-A645)]×V/1000×W

Extraction of chlorophyll and carotenoid by using DMSO by using Arnon, 1949 method
Test tube having 5ml of DMSO were preheated to 65ºC in water bath for 24, 48 hrs. This time was judged satisfactory for full decolourisation of tissues.

Cooling at room temperature was followed for 30 min, filtration and absorption measured at 480nm, 645nm, 663nm by using above formula.

Result and Discussion

Greens are important sources of protective food. It indicates healthy nature of plant and prevents it from diseases also highly beneficial for the maintenance of good health. In this study lentil plant leaves were used to estimate the chlorophyll content.

In this present study bio control and fungicide improve the chlorophyll content over control. *T. viride* shows maximum increase in chlorophyll content ("a", "b" and total) and carotenoid followed by P. fluorescence and carbendazim. Thiram shows less improvement. The results of chlorophyll and carotenoid concentrations in lentil leaves are presented in Table 1 and 2.

DMSO extracted much larger concentrations of chlorophylls when compared with80% acetone. DMSO extracted was considered to be the best chlorophyll extractor because it provided the best stability for extracts (Tait and Hik 2003) [5]. Richardson *et al.* 2002 [4] observed the same observation. He extracted, estimated and determined chlorophyll and different pigment in black gram leaves using different methods, the main pigments are chlorophyll a, b and pheophytin and carotenoid.

Table 1: Estimation of Chlorophyll content in leaves treated with bio control agent and fungicide using DMSO

Treatment	Wavelengths (nm)			Chl "a"	Chl "b"	Total chl	Carotenoid
	480	645	663				
Control	1.339	0.779	1.968	1.475	0.794	2.269	0.209
T. viride	2.568	1.345	3.213	3.718	1.576	5.293	1.920
P. fluorescence	1.682	0.914	2.317	2.696	1.008	3.704	1.720
Carbendazim	1.600	0.873	2.309	2.697	0.972	3.615	0.364
Thiram	1.513	0.827	2.114	1.911	0.918	3.268	0.552
CD at 5%	0.17	0.042	0.132	0.322	0.118	0.726	0.217

Table 2: Estimation of Chlorophyll content in leaves treated with bio control agent and fungicide using acetone.

Treatment	Wavelengths (nm)			Chl "a"	Chl"b"	Total chl	Carotenoid
	480	645	663				
Control	0.840	0.332	1.031	1.210	0.274	1.497	0.065
T. viride	1.172	0.451	1.389	1.590	0.382	2.024	0.114
P. fluorescence	1.002	0.358	1.166	1.380	0.300	1.658	0.084
Carbendazim	0.929	0.356	1.101	1.301	0.286	1.602	0.078
Thiram	0.921	0.349	1.097	1.300	0.298	1.584	0.085
CD at 5%	0.068	0.015	0.043	0.076	0.019	0.076	0.018

Conclusion

Chlorophyll from lentil leaves was extracted and estimated. The concentration of chlorophyll may vary with different wavelength. The equations used to calculate Chl concentration might also influence the results because the absorption spectra of Chl "*a"* and Chl "*b"* are different in DMSO and acetone.

Ethanol has many advantages (It is practical, safe and inexpensive) as a solvent for chlorophyll extraction. However, present study showed that it always underestimates the concentration of pigments in leaf extracts, and its use should be carefully evaluated. Acetone can be recommended, but is highly flammable, volatile, causes headaches and skin irritation; it is therefore an impractical solvent for use outside the research laboratory. The better of the two solvents used in this study, appears to be dimethylsulphoxide, because it does not require maceration, centrifugation or filtration.

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