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COMPARATIVE ACCOUNTS OF CHLOROPHYLL CONTENT, ANTIOXIDANT AND PHENOLIC CONTENTS IN *COLEUS FORSKOHLII* AND *ANDROGRAPHIS PANICULATA*

Nand Kumar Tripathi[1], Manish Kumar Upadhyay[2]*, Smt. Madhuri Tripathi[1]

[1]Institute of technology, Korba (CG), India.
[2]Dr C V Raman University, Kota, Bilaspur (CG), India.

ABSTRACT

Acidic soil of Chhattisgarh is not suitable for the proper growth of plant species. Amended soil by fly ash Prashank K. Sarangi et al have reported that amylase and invertase in soil (mg glucose. g^{-1} soil h^{-1}) and soil protease activity (µg tyrosine $g^{-1}.h^{-1}$), soil respiration (gm $CO_2M^{-2}h^{-1}$) and chlorophyll content mg. g^{-1} fresh tissue have raised. L.K.Thetwar et al have worked on the effect of fly ash and plant hormones on soil metabolic activities.

Key words: Chlorophyll Content, Antioxidant and Phenolic Contents, *Coleus forskohlii* and *Andrographis Paniculata*.

INTRODUCTION

In the present work on Coleus forskohilii and *Andrographis paniculata* flyash treated soil we observed exuberant growth of *C. forskohlii* and *A.paniculata*. Photographs are enclosed in the next pages of the whole plant, their inflorescence fruits and seeds. Fruits of *C. forskohlii* are very small and seeds are shining black in colour and that of *A.paniculata* are very small cylindrical and dull yellow and size is smaller than mustard seeds, in taste both the seeds are tasteless [1-3].

Chlorophyll Estimation

Chlorophylls are the essential components for photosynthesis. They occur in chloroplasts as green pigments in all photo synthetic plant tissues. They are loosely bound to proteins but are readily extracted in organic solvents such as ether or acetone.

MATERIALS AND METHODS

Dilute analytic grade acetone to 80% acetone. Instrument spectrophotometer.

Procedure

Leaves are grind to a fine pulp with the addition of 20 ml. of 80% acetone. Centrifuge (5000 rpm) for five minutes. Transfer the supernatant to a 100 ml. volumetric flask. Repeat the procedure. Collect the washings in the volumetric flask. Make up the volume to 100 ml. with 80% acetone.

Read the absorbance of the solution at 645, 663 and 652 nm against the solvent (80% acetone) blank.

Calculation

It is done as described below :-

(1) mg chlorophyll – a/g tissue as :
12.7 (A663) – 2.69 (A615) x (V1)/(1000xW)

(2) mg chlorophyll – b/g = 22.9 (A645 – 4.68 (A663) x (V)/(1000xW)

(3) mg of total chlorophyll g/tissue =20.2 (A645) + 8.02 (A663) x (V)/(1000xW)

A = Absorbance at specific λ
V = Final volume of chlorophyll extract in 80% acetone
W = Fresh weight of tissue extracted [4]

Corresponding Author :- **Manish Kumar Upadhyay** Email:- Man_bsp@rediffmail.com

ESTIMATION PHENOLIC CONTENTS

Phenols are aromatic compounds with hydroxyl groups are wide spread in plant kingdom. They occur in all parts of the plants.

Principle of estimation

Phenols react with phosphomolybdic acid in folin ciocalteau reagent in alkaline mediums and produce blue coloured complex (molybdenum blue).

Materials

80% Ethanol
Folin – Ciocalteau Reagent
Na_2CO_3 – 20%
Standard (100 mg catecol in 100 ml water)
Dilute 10 times for a working standard.

Procedure

Weigh 0.5 to 1 g of the sample and grind it with pestle and mortar in 10 times volume of 80% ethanol in 10 time volume of 80% ethanol. Centrifuge of 10000 rpm for 20 minutes. Evaporate the super-natant liquid to dryness. Dissolve the residue in a known volume of distilled water (5 ml.). Pipette out different aliquots (0.2 to 2 ml.) in to test tubes. Make up the volume in each tube to 3 ml with water. Add 0.5 ml of folin – ciocalteau reagent. Then 2 ml of 20% Na_2CO_3 solution to each tibe. Mix thoroughly. Place the tube in boiling water for exactly one minute. Cool. Measure the absorbance at 650 nm against a reagent blank. Prepare a standard curve with the standard curve find out the concentration of phenols in the test sample.

Folin – Ciocalteau Reagent

Reflux gently for 10 hours a mixture consisting 100 g. sodium tungstate, 25 g. Sodium molybdate, 700 ml water, 50 ml. of 85% phosphoric acid and 100 ml. of Conc. HCl in 1.5 L flask. Add 150 g. Lithum Sulphate, 50 ml. water and a few drops of bromine water. Boil the mixture for 15 minutes without condenser to remove excess bromine. Cool. Dilute to 11 filter. The regent should have no greenish tint.

RESULTS AND DISCUSSION

Results of chlorophyll content and phenolic contains have been displayed in the previous pages (Table No. 1, 2, 3 and 4). It is an important point to note that the analysis of variability for qualitative and quantitative traits depends upon the soil of the area. [5].

Table 1. Chlorophyll estimation in *C. forskohlii*

S.No.	Sample No.	Symbol used	Chlorophyll a.mg/g	Chlorophyll a.mg/g	Total
1	Plant when grown in plain soil	[A]	3	0.32	3.32
2	When grown in 5% Fly ash treated soil	[B]	3.2	0.4	3.6
3	When grown in 10% flyash treated soil. When plants were sprayed with Indole acetic acid and Gibberellic acid periodically	[C]	3.5	0.5	4.00

Table 2. Chlorophyll estimation in *A.paniculata*

S.No.	Sample No.	Symbol used	Chlorophyll a	Chlorophyll b	Total
1	In plain soil	[A]	2.5 mg/g	0.3 mg/g	2.28 mg/g
2	In soil + 5% Fly ash	[B]	2.5 mg/g	0.4 mg/g	3.7 mg/g
3	In soil + 10% Fly ash + Hormones sprayed	[C]	2.9 mg/g	0.45 mg/g	3.9 mg/g

Since *C. forskohlii* has large and glabrous leaves with 3 times larger surface area it has larger amount of Chlorophyll.

Table 3. Phenols in the tuber of *C. forskohlii*

S.No.	**Phenols in the tuber of *C. forskohlii***	
	[A] 1 mg/100 mg	tuber 20 mg/GAE/gm
	[B] 1.8 mg/100 mg	tuber 22 mg/GAE/gm
	[C] 2.5 mg/100 mg	tuber 29 mg/GAE/gm

Table 4. Phenols in *A.paniculata* in the whole plant

S.No.	**Phenols in *A.paniculata* in the whole plant v (i.e. stem, fruit or leaf)**	
	[A] 1 mg/100 mg	Whole plant 37± 4.26mg/GAE/gm
	[B] 1.8 mg/100 mg	Whole plant 38± 4.26mg/GAE/gm
	[C] 2.5 mg/100 mg	Whole plant 40± 4.26mg/GAE/gm

Comparative development of Coleus forskohlii and Andrographic paniculata after "C" treatment [i.e.80% soil + 20% Flyash + foliar spray on plants of I.A.A.and G.A.]

[1]

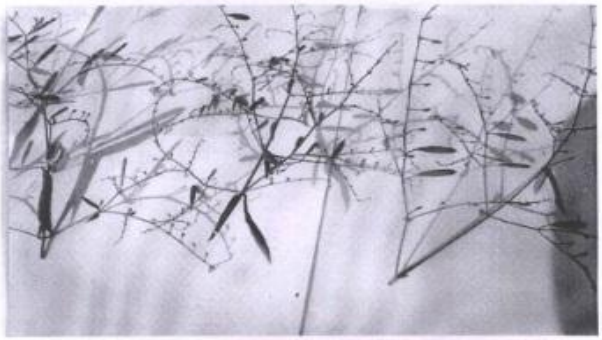
[2]

Comparative development of Coleus forskohlii and Andrographic paniculata after "C" treatment

A. paniculata mature plant with fruits and seeds

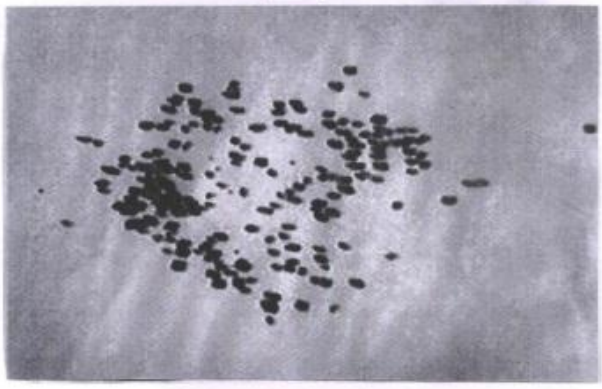
seeds

[3]



Seeds

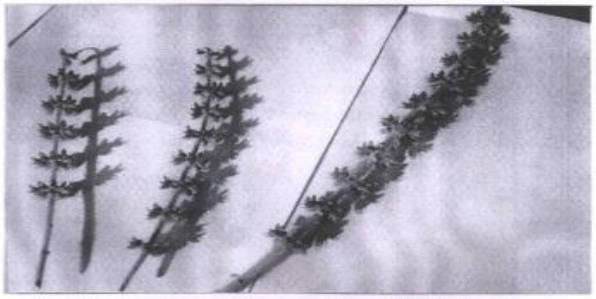
[4]



Coleus forskohlii

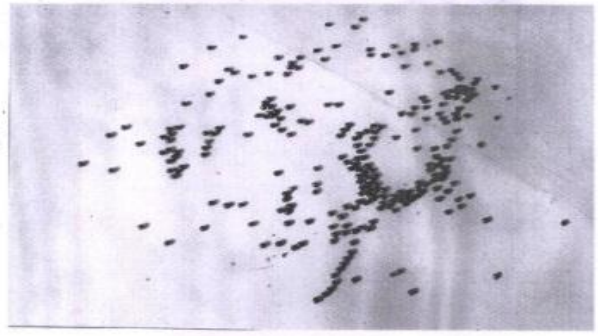
Comparative development of inflorescence (dried) with seeds in side

[5]



Seeds below the size of mustard seeds, black in colour

[6]



A variety of plant secondary metabolites in the both medicinal plants have been reported to act as an antioxidants. Plant leaves one sink for the production of these bio active compounds to the antioxidant potential of different plant species. [6]. Despite chlorophyll content of *C. forskohlii* is higher as compated to the *A. Peniculata* it has lower phenolic contents. But *C. forskohlii* is well known for new diterpenoides. They display blood pressure lowering and cardioactive properties. [7].

Many diterpeneiods are present in *C. forskohlii*. They help regulate heart muscles function. Raise insulin secretion and excellent cardiotonic, antiaging and arterial relaxant. [9] [10]. Neo andrographolide = a diterpene lactone isolated from *A.paniculata* is known for its anti inflamanatory activities. [11]. Anti cancer and apoptotic activities of andrographilide is well known. [12]. To summarise the paper, the significance of chlorophyll estimation is its contribution to absorb light energy for production of bioactive compounds, absorption of CO_2 and release of oxygen. In both plant species it is there. The phenolic contents, their anti-oxidant properties, various diterpenenoids and their significance are common.

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