



## Variable Rates of Primary and Secondary Metabolites during Different Seasons and Physiological Stages in *Convolvulus*, *Datura* and *Withania*.

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### ABSTRACT

In the present investigation attempts have been made to investigate the rate of plant metabolism during different seasons (Rainy /Winter/Summer) and physiological stages (Seedling, vegetative / flowering / maturity). Variable amount of metabolites have been found in the species with change in season and physiological stages. Investigations provided an idea of time of harvest of individual drug with optimum concentration of active principles like alkaloids, polyphenols, organic acids etc. Medicinal plants worked out include *Convolvulus*, *Datura*, and *Withania*.

**KEY WORDS:** *Convolvulus*, *Datura*, *Withania* leaf pigments, proline, protein, alkaloids, phenols, growth seasons and growth stages

### INTRODUCTION

Rate of assimilation, translocation and its utilization indicates the growth potential of plants under prevailing conditions. Limits of abiotic stress vary with the stage of development. Period of accumulation, nature and quality of metabolism in the plants is variable with the season. Maximum production of metabolites depends on age and growth phase of the plant. Harvesting of crude drugs with higher concentration of active principle is prerequisite in preparation of efficacious drugs.

*Convolvulus microphyllus* Sieb. ex. Spreng. (Convolvulaceae) is a prostrate herb. It is one of the important sources of ayurvedic drugs and is commonly known as Shankhapushpi. The leaves contain an alkaloid evolvin with significant stimulant activity. The drug is used as brain tonic. In the present era of competition Shankhapushpi syrup has gained prime position in the minds of primary and secondary school children. Its efficacy in promoting intellect has been established very well [1].

*Datura metel* L. (Solanaceae) leaves contain about 0.5 per cent of alkaloids, chiefly scopolamine (hyoscine) with traces of hyoscyamine and atropine [2]. The seeds contain about 0.2 per cent alkaloids. Atropine has a stimulant action on the central nervous system and depresses the nerve endings to the secretory glands and plain muscles. Hyoscine lacks the central stimulant action of atropine; its sedative properties enable it to be used in the control of motion sickness. Atropine and hyoscine are used to a large extent in the ophthalmic practice to dilate the pupil of the eye [3]. Atropine is well known antidote.

*Datura* is one of the Kayakalpa medicines used for rejuvenation therapy [4]. It is reported to cure bronchitis [5] and skin diseases [6] and hydrophobia [7].

Roots of *Withania somnifera* Dunal. (Solanaceae) contain several pyrazole alkaloids and withanoloides. Its sedative, tonic, stimulant and aphrodisiac properties are well known to ayurveda. It restores loss of memory [1]. Present investigation was aimed to understand an optimum time of harvest with maximum amount of metabolites.

### MATERIALS AND METHODS

Plant material for *Convolvulus* and *Withania* were collected from natural habitat of the college campus in different seasons and at different physiological stages during 2007 to 2009. However in case of *Datura metel* L.

plants were raised by sowing the seeds in circular cement pots of 40 cm x 30 cm size for three consecutive seasons. Seeds obtained from Dhanwantari Udyan (Medicinal Plant Garden), Mahatma Phule Agricultural University Rahuri

(M.S.) were used to raise the plants. The pots were filled with 10 kg of sun dried garden soil and well rotten compost in the ratio of 3:1. Cultivation was designed in quadruple. About 10 seeds of uniform size were sown equidistantly at an equal depth. Pots were irrigated on the basis of water holding capacity of the soil. In each pot plants were thinned out 15 days after germination and three well-established and uniform plants were kept at equal distance from one another. The leaf samples were collected at different developmental stages such as seedling, vegetative, flowering and maturity (senescence). For polyphenols, protein and alkaloid estimation, leaf material was initially sun dried for the period of one week. After sun drying the leaf material was oven dried at  $70^{\circ}\text{C} \pm 2^{\circ}\text{C}$  till the constant weight obtained [8]. Chlorophyll and proline content were estimated by using fresh fully expanded leaves by the method of [9, 10] respectively. Values for leaf proline were converted for dry weight on the basis of moisture content. Protein contents were estimated by the method of [11]. Alkaloids in *Datura* were estimated by the method of [12, 13] in others. Polyphenols were estimated by the method of [14]. All the estimations were done in triplicate. The data obtained was statistically analysed according to "analysis of variance" [15].

## RESULTS AND DISCUSSION

Results on concentration of leaf pigments, proline, protein content, total alkaloids and phenols during different seasons and growth stages are depicted in Table 1 to 6.

Chlorophyll, proline, alkaloids and phenol concentration was highest in *Convolvulus* during summer season at flowering stage (Table 1 and 2). However protein concentration in it was recorded highest during winter at flowering stage. In *Withania* chlorophyll and polyphenols both were maximum in winter during vegetative stage (Table 5 and 6).

**Table 1 Seasonal variations in physiological parameters of *C. microphyllus***

Parameters	Seasons		
	Summer	Rainy	Winter
Total chlorophylls (mg/100gm F.Wt)	189.7 $\pm$ 1.3	161.1 $\pm$ 3.2	172 $\pm$ 3 $\pm$ 2.4
Proline (mg/100gm F.Wt)	165.1 $\pm$ 1.9	160.7 $\pm$ 1.9	159.4 $\pm$ 3.9
Proteins (gm/100gm D.Wt)	06.35 $\pm$ 1.4	11.69 $\pm$ 0.2	19.43 $\pm$ 0.2
Total Alkaloids (mg/100gm D.Wt)	2.34 $\pm$ 0.3	1.32 $\pm$ 0.1	2.12 $\pm$ 0.2
Polyphenols (mg/100gm D.Wt)	64.7 $\pm$ 3.0	42.3 $\pm$ 1.3	47.2 $\pm$ 0.5

In *Datura* maximum chlorophyll contents were recorded in rainy season with the highest concentration in flowering stage (Table 3 and 4). Minimum values were recorded in summer. Increase in chlorophyll in monsoon indicates high efficiency of photosynthetic apparatus.

Proline content was maximum at maturity stage during summer followed by winter season. Minimum values were recorded in rainy season. Proline is excellent stress indicator. The accumulation of free proline depends on the type and intensity of stress [16]. Water deficit is believed to stimulate the synthesis of an osmoregulator proline during summer [17].

**Table 2 Variations in physiological parameters of *C. microphyllus* under different growth stages**

Parameters	Growth Stages		
	Seedling (25Days)	Vegetative (65Days)	Flowering (115Days)
Total chlorophylls (mg/100gm F.Wt)	43.8 $\pm$ 1.2	62.6 $\pm$ 2.9	97.2 $\pm$ 3.1
Proline (mg/100gm F.Wt)	85.4 $\pm$ 2.1	61.3 $\pm$ 1.8	118.9 $\pm$ 2.4
Proteins (gm/100gm D.Wt)	10.7 $\pm$ 1.3	3.87 $\pm$ 0.9	13.92 $\pm$ 1.7
Total Alkaloids (mg/100gm D.Wt)	0.807 $\pm$ 0.2	1.68 $\pm$ 0.3	1.92 $\pm$ 0.1
Polyphenols (mg/100gm D.Wt)	22.1 $\pm$ 0.1	27.6 $\pm$ 0.7	44.2 $\pm$ 1.2

**Table 3 Seasonal variations in physiological parameters of *D. metel***

Parameters	Seasons		
	Summer	Rainy	Winter
Total chlorophylls (mg/100gm F.Wt)	93.77±1.6	119.46±3.1	101.86±2.4
Proline (mg/100gm F.Wt)	162.28±4.2	138.49±3.9	143.33±6.0
Proteins (mg/100gm D.Wt)	417.00±3.8	366.82±5.1	384.00±4.3
Total Alkaloids (mg/100gm D.Wt)	378.12±5.7	432.00±5.2	406.00±4.6
Polyphenols (g/100gm D.Wt)	1.13±0.24	1.73±0.29	1.60±0.20

**Table 4 Variations in physiological parameters of *D. metel* under different growth stages**

Parameters	Growth Stages			
	Seedling (30Days)	Vegetative (45Days)	Flowering (75Days)	Maturity (105Days)
Total chlorophylls (mg/100gm F.Wt)	56.19±1.2	71.88±1.9	99.37±2.4	68.14±1.3
Proline (mg/100gm F.Wt)	71.00±2.1	89.70±1.7	93.00±3.9	113.00±4.1
Proteins (mg/100gm D.Wt)	292.00±1.71	314.67±2.1	362.71±1.7	316.00±3.2
Total Alkaloids (mg/100gm D.Wt)	*	102.72±1.9	457.11±5.3	402.30±3.8
Polyphenols (g/100gm D.Wt)	0.68±0.08	1.1±0.68	1.32±0.13	0.98±0.23

**Table 5 Seasonal variations in physiological parameters of *W. somnifera***

Parameters	Seasons		
	Summer	Rainy	Winter
Total chlorophylls (mg/100gm F.Wt)	76.12 ± 1.6	103.46 ± 3.1	114.08 ± 2.4
Polyphenols (mg/100gm D.Wt)	218.44 ± 1.3	270.61 ± 3.2	292.04 ± 1.7

Maximum protein contents were recorded in summer at flowering stage. Minimum values were recorded in rainy season. Water stress causes both reductions in the role of protein synthesis as well as changes in the type of proteins produced. Formation of free amino acids in *D. metel* may have lead to the synthesis of new desired proteins during summer.

Maximum alkaloid contents were recorded in rainy season at flowering stage followed by winter (Table 3 and 4). Minimum values were recorded in summer. Rainy season has positive impact on plant growth as well as the formation of alkaloids. Alkaloid synthesis increases with progressive maturity. It reaches to highest concentration at flowering stage. In the present work alkaloid synthesis could be recorded in the order Flowering stage> Maturity stage> Vegetative stage. Seedling stage has shown no synthesis of alkaloids. Most alkaloids are formed in young and actively growing tissues [18]. Results of alkaloid accumulation are in consonance with [19] who reported maximum accumulation of alkaloids in the leaves of *D. innoxia* during flowering stage. [20] reported maximum accumulation of alkaloids and phenols in *Asparagus racemosus*, *Boerhavia diffusa* and *Sida cordifolia* at flowering stage during summer and no alkaloids accumulation in seedling stage.

**Table 6 Variations in physiological parameters of *W. somnifera* under different growth stages**

Parameters	Growth Stages		
	Seedling (30Days)	Vegetative (45Days)	Flowering (75Days)
Total chlorophylls (mg/100gm F.Wt)	28.04 ± 0.8	39.56 ± 1.2	31.41 ± 1.7
Polyphenols (mg/100gm D.Wt)	61.10 ± 1.4	83.11 ± 1.6	79.10 ± 1.9

Observations are mean of three determinations. ±Indicates standard deviation.

\*No formation of alkaloids.

Highest concentration of polyphenols was recorded in rainy season during flowering stage followed by winter and lowest in summer. The results are contradictory to [21] who observed increased phenolic compounds in sorghum due to water stress. The stage of growth of the plant has an impact on phenolic contents.

From the present study it is concluded that growth stages and seasons show effect on eco-physiological parameters. In *C. microphyllus* summer season was found to be the most favorable during which chlorophyll, proline and phenols showed highest concentration during flowering stage. Favorable climatic conditions are believed to stimulate the secondary metabolite synthesis in *D. metel* L. Rainy season was found to be most favorable for maximum production of leaf pigments, alkaloids and polyphenols. In *Withania* vegetative stage stimulates chlorophyll and phenol synthesis during winter.

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