

Factors Influencing Secondary Metabolite Synthesis in Medicinal and Aromatic Plants

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ABSTRACT: Secondary metabolites are organic compounds produced by plants in smaller amounts which are not concerned with the growth, photosynthesis, reproduction or any other primary functions. These molecules are known to play a vital role in the adaptation of plants to their environment, serve as defense and signal compounds and indirectly help in pollination. Secondary metabolites include alkaloids, terpenes, phenols, glycosides, saponins, gums, oleoresins, etc. which are potential sources of pharmaceuticals, agrochemicals, flavours, fragrances, colours, botanicals, food additives etc. They are considered as active principles of medicinal and aromatic plants.

The global market for traditional medicines was estimated at US\$ 83 billion annually in 2008 and increasing at the rate of 5-15 per cent annually. This is an indication of possible growing demand for herbal drugs in coming years. Several factors like stress factors, genetic factors, developmental stages of plant, dates of sowing, nutrient levels, growth regulators, mutagens etc. influence on the secondary metabolites in plants.

Stress conditions resulted in the higher secondary metabolite accumulation which play a major role as defense mechanism in plants. Seasonal variations alter the secondary metabolite content in plants. Planting at the right time with optimum fertilizer dose resulted in highest yield of these compounds. Hence, to meet the increasing demand, all the factors have to be considered to enhance the production of the secondary metabolites prior to commercial cultivation of these crops.

Keywords: Secondary metabolites, defense mechanism, plant stress and Higher yield.

INTRODUCTION

Secondary metabolites are organic chemicals produced by the plants in minute quantities which are not considered with the growth, photosynthesis, reproduction or any other primary functions of plants.

- Adam Lonicer (1528-1586): one of the pioneer research workers of essential oils-gave their nature and importance.
- More than 1,20,000 substances has been identified in different plant spp.
- These chemicals are the potential sources of pharmaceuticals, agrochemicals, flavours, fragrances, colours, biopesticides and food additives.
- These are considered as active principles of medicinal and aromatic plants.

- They Occur in special, differentiated cells like vacuoles, resin ducts, laticifers, trichomes or in cuticle.
- Sites of synthesis and storage often differ.

Alkaloids	Plant species	Site of synthesis	Site of accumulation
Nicotine	<i>Nicotiana</i>	Roots	Whole plant
Diosgenin	<i>Dioscorea</i>	Leaf	Tubers
Caffein	Coffee	Bark	Berries
Ajmalicine	<i>Catharanthus</i>	Roots	Roots
R-ginsenoside	<i>Panax</i> spp.	Roots	Roots
Tropane alkaloids	<i>Datura</i> , Henbane	Roots	Leaf
Glycyrrhizin	<i>Glycyrrhiza</i>	Rhizomes	Rhizomes

- Alkaloids, terpenes, phenols, glycosides, saponins, gums and oleoresins etc. come under secondary metabolites.

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- These molecules are known to play a vital role in the adaptation of plants to their environment. They serve as defense and signal compounds and indirectly helps in pollination (colors of flowers).
- **Plant defense mechanism-Ex:** L-DOPA in *Mucuna spp.* against *Bruchid beetle*.
- **Indirectly helps in pollination-colors of flowers-Ex:** Delphinidin 3,5- diglucoside-attracts bumble bees and humming birds.
- **Acts as a signaling agents- plant hormones-Ex:** Gibberelins enhance seed germination and stem elongation.
- **Antifungal agents-Ex:** Catechol in onion against *Colletotrichum circinans*.

DIFFERENCE BETWEEN PRIMARY AND SECONDARY METABOLITES

Primary metabolites	Secondary metabolites
Have metabolic functions essential for plant growth and development.	Don't have apparent functions involved in plant growth and development.
Produced in every plant.	Produced in different plant in specific tissues, cells or developmental stages and different plant parts.
Fairly large amounts Produced in the cells.	Produced in small amounts.
Produced for energy synthesis.	Specific purposes.
Major biochemical pathways and synthesis.	Subsidiary pathways from primary metabolites.
Essential for life processes.	Non essential for basic life processes.
Ex: Carbohydrates, Proteins, Lipids.	Ex: Alkaloids, terpenes, phenols, etc.

IMPORTANCE OF SECONDARY METABOLITES

The world market for herbal drugs are increasing at the rate of 5-15% annually, which is an indication of possible growing demand for herbal drugs in coming years.

Even though plants produce these chemical compounds in minute quantities, but still they are preferred because of their world wide applications in different fields like:

- Potential sources of plant based drugs. Ex: Quinine-antimalarial drug.
- Used in many cosmetic industries.
- Constituents of perfumes.
- Coloring agents.

- Insecticides and others.

MEDICINAL SIGNIFICANCE

Compound	Plant source	Therapeutic use
Codeine and morphine	Opium poppy	Analgesic
Atropine Hyoscyamine	Belladonna <i>Datura, Hyoscyamus</i>	Anticholinergic
Caffein	Coffee, tea, cocoa	Stimulant
Vincristine, vinblastine	periwinkle	Anticancer
Nicotine	Tobacco	Smoking cessation
Quinine	<i>Cinchona</i>	Antimalarial
Resepine	<i>Rauwolfia</i>	Reduce hypertension
Quinidine	<i>Cinchona spp</i>	Cardiac depressant

Aroma principles

Common name	Botanical name	Active principle	Uses
Rose geranium	<i>Pelargonium graveolens</i>	Geraniol, rhodinol	Perfumes, power, creams, body lotions.
Japanese mint	<i>Mentha arvensis</i>	Menthol, carvone, linalool	Scenting in the supari
Bergamot mint	<i>Mentha citrata</i>	carvone	Soaps and detergents
Lemon grass	<i>Cymbopogon flexuosus</i>	Citral, farnesol	Starting material for ionones and vitamin-A manufacturing.
Patchouli	<i>Pogostemon patchouli</i>	pachouliol	Fixative property
Indian basil	<i>Ocimum basilicum</i>	Methyl chevicol	Flavouring foods
Rosemary	<i>Rosemarinus officinalis</i>	Camphene, cineol.	Anti cancer and antioxidant property
Davana	<i>Artemisia pallens</i>	Hydrocarbons	Delicate fragrance for floral decortion, bouquets, cosmetics.

Classification of secondary metabolites

- **Nitrogen containing compounds**
Ex: Alkaloids, glucosinolates
- **Phenolics**
Ex: Lignins, flavonoides, tannins, coumarins etc.
- **Terpenoids**
Ex: Monoterpenes, sesquiterpenes, diterpenes & triterpenes, sterols etc.

Different Factors Include

- I. Stress conditions.
- II. Plant factors.
 - (a) Genetic factors.
 - (b) Developmental stages of the herb.
- III. Agronomic practices.
 - (a) Season of planting/ harvesting.
 - (b) Cultural practices.
- IV. Post harvest practices.
- V. Biotechnological approach.

STRESS CONDITIONS

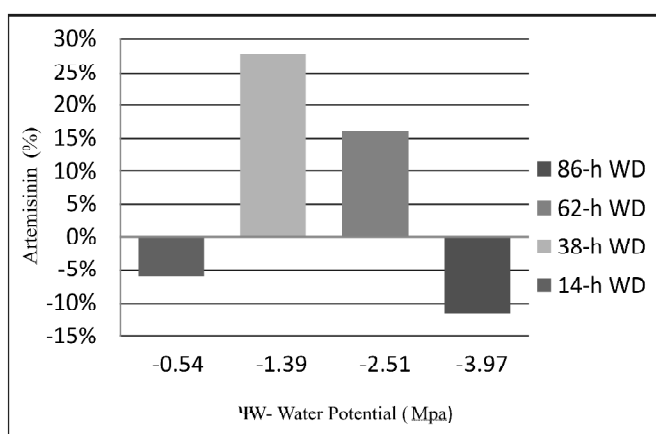


Figure 1 : Water deficit effect on the accumulation of artemisinin in *Artemisia annua* L. Marchese *et al.*, 2010, Brazil.

Marchese *et al.* (2010) reported that, the artemisinin content was highest in 38 hour water deficit treatment which is 28% more compared to the irrigated control followed by 62 hour water deficit treatment which recorded 16% more compared to irrigated control. Whereas, 14 hour water deficit and 86 hour water deficit resulted in 6% and 11.7% less artemisinin than the control. This increase in 36 hour treatment may be due to the fact that the growth decreases under moderate water deficit, while photosynthesis is still continuing at the same rate. Thus, the excess photoassimilates, used in small quantity for growth, would be redirected towards secondary metabolism such as artemisinin biosynthesis. Even, the drought induces changes in gene expression which increases metabolite production. They concluded that, lack of irrigation one or two days before crop harvest induce a moderate water stress condition and lead to a significant increase in artemisinin without affecting biomass accumulation.

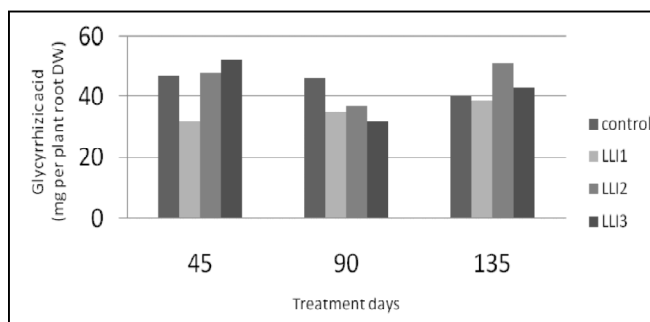


Figure 2: Glycyrrhizic acid content per plant root of *Glycyrrhiza uralensis* under low light intensity (LLI) stress on the 45th, 90th and 135th day after LLI treatments. Hou *et al.*, 2010, China.

Control = natural light, LLI1= 200 $\mu\text{mol m}^{-2}\text{s}^{-1}$, LLI2= 100 $\mu\text{mol m}^{-2}\text{s}^{-1}$, LLI3= 50 $\mu\text{mol m}^{-2}\text{s}^{-1}$ DW=dry weight.

Hou *et al.* (2010) reported that, there is no significant increase in total content of glycyrrhizic acid per plant root upto 90 days after LLI treatments. But, after 135 days under LLI conditions, highest glycyrrhizic acid content per plant root was resulted under an irradiance of 100 $\mu\text{mol m}^{-2}\text{s}^{-1}$ (27.6 %), followed by LLI 3 treatment (9.7 %). This indicates that low light stimulated the biosynthesis of secondary metabolites. This suggested that, low light intensity and spectrum were regarded as environmental stimuli for production of secondary metabolites.

Table 1
Effect of abiotic metal stress on diosgenin yield in *Dioscorea bulbifera* L. cultures. Narula *et al.*, 2005, New Delhi.

CuSO_4 (μM)	Proline ($\text{mg g}^{-1}\text{fw}$)	Protein ($\text{mg g}^{-1}\text{fw}$)	Diosgenin ($\text{mg g}^{-1}\text{fw}$)
00	1.49 \pm 0.01 ^d	7.42 \pm 0.08 ^e	1.16 \pm 0.00 ^d
25	1.81 \pm 0.09 ^c	10.77 \pm 0.09 ^d	2.59 \pm 0.00 ^c
50	2.50 \pm 0.02 ^b	12.93 \pm 0.06 ^c	5.78 \pm 0.05 ^b
75	2.70 \pm 0.02 ^a	13.50 \pm 0.07 ^b	9.59 \pm 0.02 ^a
100	2.77 \pm 0.01 ^a	14.65 \pm 0.04 ^a	1.00 \pm 0.00 ^e
LSD (0.01)	0.106	0.434	0.09

Narula *et al.* (2005) reported that, as the concentration of CuSO_4 in the medium increases proline content increases from 1.49 \pm 0.01 in control to 2.77 \pm 0.01 in 100 μM concentration of CuSO_4 indicating that the stress has been induced due to the supplementation of Cu to the medium. Diosgenin yield gradually increases as the concentration of Cu increases from 1.16 mg/ g dry weight at control to 9.59 mg/ g dry weight at 75 μM concentration of CuSO_4 due to the metal stress but higher concentration of 100 μM of CuSO_4 caused a decline in the diosgenin yield. This is because at higher concentration Cu becomes toxic.

PLANT FACTORS

- Genetic factors
- Developmental stages of the plant

GENETIC FACTORS

Table 2
Evaluation of *Mucuna utilis* genotypes for seed and L-DOPA yield.

Mamatha *et al.*, 2008, Bangalore.

Genotypes	Seed yield (Kg/ha)	L-DOPA (%)	L-DOPA yield (Kg/ha)
IIHR MP 01	944.2	5.21	49.19
IIHR MP 02	1311.7	6.61	86.70
IIHR MP 03	539.8	5.01	27.04
IIHR MP 05	1052.1	6.16	43.35
IIHR MP 06	1012.1	4.12	41.69
IIHR MP 08	521.4	4.22	22.00
IIHR MP 09	1808.8	5.11	92.39
IIHR MP 10	957.0	5.44	52.07
IIHR MP 11	1119.0	5.14	57.52
IIHR MP 16	463.0	5.34	24.72
IIHR MP 17	497.6	4.11	20.45
IC 16993/ A	505.7	4.32	21.85
IC 33243	502.1	4.44	22.29
S.Em±	25.1	0.29	3.49
CD at 5%	76.8	0.90	10.68

Mamatha *et al.* (2008) studied 13 IIHR genotypes and 2 IC genotypes. They reported that the L- DOPA content in seed samples of *Mucuna utilis* genotypes varied from 6.61 % in IIHR MP 02 to 4.11 % in IIHR MP 17. This is due to the variation of precursor compounds of L-DOPA present in the seeds. Whereas, the highest L- DOPA yield was recorded in IIHR MP 09 (92.39 Kg/ha) which is due to the highest seed yield (1808.8 Kg/ha) and lowest L- DOPA yield was recorded in IIHR MP 17 (20.45 Kg/ha) which is due to low L- DOPA content (4.11 %) and lower seed yield (497.6 Kg/ha).

Ravi *et al.* (2006) studied 17 genotypes and reported that the highest alkaloid content was found in MG- 13 (0.23 %) and lowest in MG- 6 genotype (0.05 %) with an average of 0.1407 %. This is due to the genetic make-up of the genotypes. The highest alkaloid yield was recorded in MG- 14 (12.26 kg/ ha) which is due to more alkaloid content (0.21 %) and high herbage yield and the yield was observed in MG- 6 (1.55 Kg/ha) which is attributed to lowest alkaloid content and less herbage yield.

Table 3
Performance of *Solanum nigrum* genotypes for different morphogenetic traits, yield and quality attributes.
Ravi *et al.*, 2006, Bangalore

Genotype	Plant height (cm)	Number of leaves plant ⁻¹	Leaf area (cm ²) plant ⁻¹	Herbage yield (g plant ⁻¹)		Total alkaloid content (% w/w)	Total alkaloid yield (kg ha ⁻¹)
				Fresh	Dry		
MG-1	155.10	1190.50	18026.19	1586.33	209.07	0.08	6.05
MG-2	123.57	830.53	7831.18	852.47	120.93	0.074	3.31
MG-3	110.50	405.60	6123.88	847.87	129.87	0.06	2.85
MG-4	104.20	668.80	8819.43	588.73	93.47	0.10	3.46
MG-5	114.07	911.20	16412.75	843.93	130.40	0.14	6.69
MG-6	77.23	236.87	3810.57	391.33	84.53	0.05	1.55
MG-7	108.03	736.47	13030.79	830.33	119.60	0.057	2.51
MG-8	112.90	570.07	10504.39	783.37	112.53	0.120	4.96
MG-9	111.87	619.80	11160.86	919.73	149.13	0.152	8.37
MG-10	112.93	414.87	7427.62	758.07	126.73	0.21	9.83
MG-11	112.93	818.00	7958.90	888.00	154.00	0.192	10.97
MG-12	101.57	478.00	7545.03	548.33	99.60	0.18	6.58
MG-13	124.00	782.20	13922.67	934.33	140.80	0.23	11.91
MG-14	133.53	940.33	14605.02	1094.67	161.73	0.21	12.26
MG-15	126.00	895.93	6496.16	916.07	140.87	0.16	8.32
MG-16	114.43	692.80	11833.98	735.60	137.73	0.22	11.11
MG-17	123.42	930.00	7117.53	893.20	140.80	0.19	9.87
SEm±	7.80	122.21	1631.27	126.80	15.33	0.01239	0.945
CD@5%	21.61	338.76	4521.66	351.48	42.51	0.0343	2.60

DEVELOPMENTAL STAGES OF PLANT

Table 4
Effect of Leaf Age on Gymnemic Acid Content (%) in *Gymnema sylvestre* Across Seasons.

Singh et al., 2006, Bangalore.

Leaves	Summer	Rainy	Winter	Mean
Young leaves (< 2 months)	7.14	7.62	7.28	7.35
Old leaves (2-4 months)	2.98	3.06	2.86	2.97

Singh et al. (2006) reported that the younger leaves possessed the higher gymnemic acid content of 7.14 %, 7.62 %, 7.28 % during summer, rainy and winter seasons compared to the older leaves. Hence, if the herb is cultivated, the plant architecture may be so designed as that of tea plant to yield lot of young leaves so that active principle is harvested frequently at periodic intervals.

Table 5
Effect of Different Stages of Plant Growth on Recovery of L-DOPA in *Mucuna* spp.

Shivananda et al., 2009, Bangalore.

Stages of plant growth	Recovery of L-DOPA at different growth stages in <i>M. pruriens</i>							
	2001				2002			
	M.C. (Moisture content)	L-DOPA (%)	Seed yield (Kg/ha) Air DW	L-DOPA recovery (Kg/ha)	M.C.	L-DOPA (%)	Seed yield (Kg/ha) Air DW	L-DOPA recovery (Kg/ha)
At physiological maturity	76	7.8	240	18.72	78.5	7.6	264	20.06
At harvest	12	7.1	715	50.77	11.5	7.0	782	54.74
CD @ 0.05	7.2	3.30	37.5	9.54	7.8	0.52	31.89	8.61
SEm ±	2.4	0.10	12.50	3.18	2.6	0.14	10.63	2.87
Stages of plant growth	Recovery of L-DOPA at different growth stages in <i>M. utilis</i>							
	M.C. (Moisture content)	L-DOPA (%)	Seed yield (Kg/ha) Air DW	L-DOPA recovery (Kg/ha)	M.C.	L-DOPA (%)	Seed yield (Kg/ha) Air DW	L-DOPA recovery (Kg/ha)
	At physiological maturity	74.5	4.8	340	26.52	76.5	4.6	364
At harvest	12.0	4.2	1180	83.78	12.5	4.2	1354	94.78
CD @ 0.05	6.30	0.93	32.04	11.70	8.55	1.38	29.70	12.9
SEm ±	13.10	0.31	10.68	3.9	2.85	0.46	9.90	4.3

Shivananda et al. (2009) reported that L-DOPA content was significantly higher at physiological maturity stage compared to harvest stage in *Mucuna pruriens* (7.8%) and *M. utilis* (4.8%) during both the year. But, L-DOPA recovery was significantly higher at harvest than at physiological maturity in both the species during both the years which is due to higher seed yield at harvest. Hence, even though the L-

DOPA content is higher at physiological maturity, harvesting the pods at complete maturity is highly profitable than at physiological maturity stage.

Agronomic Practices

- Season of planting/ harvesting.
- Cultural practices.

SEASON OF PLANTING/HARVESTING

Table 6
Effect of Date of Sowing and Stage of Harvesting on Root Yield and Quality of Ashwagandha under Rainfed Conditions

Kubsad et al., 2008, Dharwad.

Treatments	Dry root yield (Kg/ha)	Total withanolide content (%)	Total withanolide yield (Kg/ha)	Starch content (%)
Dates of sowing(D)				
D ₁ (Aug.15)	1166 ^c	0.487 ^b	5.673 ^c	16.3 ^b
D ₂ (Aug.30)	1202 ^b	0.482 ^b	5.842 ^c	18.4 ^a
D ₃ (Sep.15)	1323^a	0.537^a	7.233^a	14.6 ^c
D ₄ (sep.30)	1239 ^b	0.528 ^a	6.618 ^b	13.2 ^d
S.Em±	16	0.006	0.116	0.07
Stage of harvesting(H)				
H ₁ (120DAS)	1049 ^c	0.468 ^c	4.906 ^c	17.9 ^a
H ₂ (150DAS)	1187 ^b	0.498 ^b	5.840 ^b	15.8 ^b
H ₃ (180DAS)	1461^a	0.560^a	8.181^a	13.1 ^c
S.Em±	14	0.005	0.100	0.06

contd. table 6

Treatments	Dry root yield (Kg/ha)	Total withanolide content (%)	Total withanolide yield (Kg/ha)	Starch content (%)
Interaction (DXH)				
D ₁ H ₁	1022 ^{fg}	0.455 ^f	4.647 ^g	20.5 ^a
D ₁ H ₂	1142 ^e	0.485 ^{df}	5.539 ^e	15.5 ^d
D ₁ H ₃	1335 ^c	0.519 ^{cd}	6.922 ^d	12.8 ^f
D ₂ H ₁	1072 ^f	0.452 ^f	4.849 ^{fg}	20.1 ^a
D ₂ H ₂	1145 ^e	0.470 ^{ef}	5.380 ^{ef}	18.3 ^b
D ₂ H ₃	1390 ^c	0.525 ^c	7.298 ^c	16.6 ^c
D ₃ H ₁	1086 ^f	0.480 ^{df}	5.213 ^{eg}	16.7 ^c
D ₃ H ₂	1260 ^d	0.510 ^{ce}	6.426 ^d	16.3 ^c
D ₃ H ₃	1622^a	0.620^a	10.059^a	10.8 ^b
D ₄ H ₁	1018 ^{fg}	0.483 ^{df}	4.915 ^{fg}	14.5 ^{de}
D ₄ H ₂	1203 ^{de}	0.527 ^b	6.337 ^d	13.1 ^e
D ₄ H ₃	1497 ^b	0.575 ^b	8.603 ^b	11.9 ^g
S.Em±	28	0.011	0.201	0.12

Means followed by the same letter/s in a column do not differ significantly by DMRT (p= 0.05)

Kubsad *et al.* (2008) reported that, the highest withanolide content (0.537%) and withanolide yield (7.233 Kg/ha) was recorded in D₃ (September 15th sown crop). This may be due to higher rainfall received during early stages of crop coupled with conducive temperature (29.9 °C) which resulted in

higher growth and higher yield. The total withanolide content and yield increases as the duration of crop increases recording the highest at 180 DAS, which is due to the prolonged growing period coupled with favourable climatic conditions. In the interaction, D₃H₃ crop sown on September 15th and harvesting at 180 DAS resulted in higher withanolide content (0.620%) and withanolide yield (10.059 Kg/ha).

Table 7
Effect of Planting Date on Yield and Essential Oil Content of Rhizomes in Sweet Flag
(*Acorus calamus*).

Anita et al., 2009, Jabalpur (M.P.).

Treatments	Plant height (cm)	Number of leaves	Leaf Area	Length of rhizome (cm)	Girth of rhizome (cm)	Essential oil (%)	Fresh yield (q/ha)	Dry yield (q/ha)
D ₁	58.30	14.60	45.30	6.50	1.20	0.25	18.40	1.80
D ₂	47.30	11.80	36.30	10.50	1.30	0.32	18.60	1.81
D ₃	43.20	10.80	31.70	14.40	1.30	0.35	19.50	1.92
D ₄	38.30	9.60	20.60	6.20	1.00	0.22	16.60	1.72
D ₅	26.00	6.50	17.70	5.20	0.70	0.13	15.20	1.51
S.Em±	6.80	0.98	13.81	2.21	0.12	0.008	0.916	0.013
CD@5%	14.83	2.14	30.11	4.83	0.27	0.019	1.997	0.029

D₁- 30th June; D₂- 15th July; D₃- 30th July; D₄- 15th August; D₅- 30th August.

Anita *et al.* (2009) reported that the essential oil % was significantly higher in D₃ (30th July) (0.35 %) followed by D₂ (15th July) (0.32 %). This is due to the maximum rhizome length and rhizome girth which is due to congenial atmospheric conditions. The lowest oil content (0.13 %) was recorded in D₅ (30th August) due to delayed planting. They concluded that planting sweet flag on 30th July resulted in highest oil content.

Bagchi *et al.* (2009) reported that, the plumbagin concentration was high during winter months from December to February recording the highest during

February (1.8 %) when the plant was in flowering stage. While the lowest plumbagin content was observed during summer in the month of June (0.1 %) when the plant is in vegetative stage. This may be due to the fact that during winter, plants exhibited lesser growth and senescence and major part of the energy is diverted to store the reserve food and plumbagin to the roots to withstand the winter. Whereas, in summer more energy is diverted for leaf formation and plant growth giving less scope for plumbagin accumulation.

Table 9
Effect of Growth and Season on Plumbagin Concentration in the Roots of *Plumbago zeylanica* (Chitrak).
 Bagchi *et al.*, 2009, CIMAP.

Collection months	Growth stages	Height of plant (cm)	Plumbagin concentration (%)
January	Bud initiation	167.7±1.64	1.6
February	Budding/ Flowering	168.4±2.13	1.8
March	Flowering/ Fruiting	168.4±3.19	1.0
April	Fruiting	168.4±2.35	1.3
May	Vegetative	168.4±1.92	1.3
June	Vegetative	170.0±2.76	0.1
July	Vegetative	170.5±2.91	0.3
August	Vegetative	170.5±1.13	1.4
September	Budding	221.3±4.03	1.3
October	Flowering	238.0±1.66	1.2
November	Flowering/ Fruiting	239.0±2.80	1.5
December	Fruiting	246.0±1.97	1.7

CULTURAL PRACTICES

Table 10
Influence of Intercropping of *Ocimum basilicum* with *Cajanus cajan* on herbage and essential oil yield
 Archna *et al.*, 2009, Jabalpur (M.P.).

Treatment details:

T ₁	<i>Ocimum</i> sole crop			
T ₂	<i>Ocimum</i> + <i>Cajanus</i> 1:1 at 50x50cm spacing.			
T ₃	<i>Ocimum</i> + <i>Cajanus</i> 1:1 at 75x35.5cm spacing.			
T ₄	<i>Ocimum</i> + <i>Cajanus</i> 1:1 at 100x50cm spacing.			
T ₅	<i>Ocimum</i> + <i>Cajanus</i> 2:1 at 50x100cm spacing.			
T ₆	<i>Cajanus</i> sole crop at 100x100cm spacing.			

Treatment	Fresh herbage yield (Kg/ha)	Essential oil (%)	Essential oil yield (L/ha)	<i>Cajanus</i> seed yield (Kg/ha)
T ₁	13273	0.64	60.67	-
T ₂	9450	0.38	29.30	1516
T ₃	7662	0.38	32.08	1191
T ₄	8417	0.43	57.79	1050
T ₅	8238	0.45	37.36	800
T ₆	-	-	-	1939
S.Em±	2.42	0.01	3.97	0.49
CD at 5%level	4.84	0.04	12.25	1.51

Archna *et al.* (2009) reported that, the essential oil % (0.64 %) and fresh herbage yield (13.3 t/ ha) and essential oil yield (60.67 lit/ ha) was found to be maximum when the crop is sown as sole crop compared to intercrop. In all the intercropping treatments no significant increase in essential oil and herbage yield was noticed. This is because in

intercropping the plant growth was stunted and show significant decrease in canopy due to crowding and competition for nutrients and water between the crops. Even the seed yield of *Cajanus* was significantly higher under its sole crop (1.93 t/ ha).

Table 11
Effect of Fertilizer Levels on Growth, Yield and Quality of *Bacopa monnieri*.
 Chandrashekar *et al.*, 2006, Bangalore.

Treatments (NPK Kg/ha)	Inter-nodal length (cm)	Bran-ches / node	Roots / node	Root/shoot ratio	Fresh herb yield (g/m ²)	Dry herb yield (g/m ²)	Bacoside yield (g/m ²)
Control	2.05	1.75	1.75	3.45	1489.20	157.92	3.79
10:10:10	2.50	1.75	2.75	4.20	1515.50	160.64	4.17
20:20:20	2.10	1.75	2.50	3.93	1672.50	177.28	3.89
40:20:20	2.92	2.25	2.30	4.23	2330.00	246.98	8.15
20:20:40	2.85	2.25	2.50	3.45	1852.50	196.36	6.45
Mean	2.48	1.95	2.50	3.90	1771.95	187.84	5.30
S.Em±	0.92	0.79	1.35	0.57	510.13	54.04	0.86
CD (p= 0.05)	0.30	0.25	0.44	0.18	165.54	17.54	0.28

Chandrashekar *et al.* (2006) reported that, the bacoside yield (8.15 g/ m²) was significantly higher when the fertilizers was applied at 40:20:20 Kg/ ha of NPK. This may be because all the growth parameters and fresh herb and dry herb yield were recorded highest at this fertilizer level. Hence, among the four fertilizer levels this was found to be superior in improving the herbage yield and bacoside content.

POST HARVEST PRACTICES

Table 13
Effect of Chopping Length on Essential Oil Yield,
Oil Content and Citral Content in lemongrass.

Vedhamurthy, 2003, Arabhavi.

Treatments	Oil yield (ml/ 2 Kg)	Oil content (%)	Citral content (%)
T ₁ -control	13.60	0.68	81.13
T ₂ -3cm chopping length	24.55	1.22	85.91
T ₃ -5cm chopping length	16.85	0.86	84.92
T ₄ -10cm chopping length	15.72	0.78	84.49
T ₅ -15cm chopping length	14.85	0.73	83.49
Mean	17.11	0.85	84.08
SEm±	0.615	0.032	1.86
CD (0.01)	2.20	0.128	NS

Vedhamurthy (2003) reported the highest oil content (1.22%) and oil yield (24.55 ml/ 2Kg) in 3 cm chopping length followed by 5 cm chopping length. Oil content gradually increases as the chopping length decreases because smaller the disintegration of the material, higher was the oil yield and its constituents. This is because cutting of lemongrass into small pieces enabled the exposure of oil glands. He concluded that smaller the chopping lengths of the grass, higher was the exposure of oil glands, leading to higher recovery of the oil with reduced distillation period.

Table 14
Effect of Storage Period on Essential Oil Yield,
Oil Content and Citral Content in Lemongrass

Vedhamurthy, 2003, Arabhavi.

Treatments	Oil yield (ml/ 2Kg)	Oil content (%)	Citral content (%)
T ₁ - control	13.60	0.68	82.32
T ₂ -12 hrs of storage of grass	14.62	0.73	84.92
T ₃ -24 hrs of storage of grass	17.25	0.86	85.82
T ₄ -48 hrs of storage of grass	18.62	0.93	85.73
T ₅ -72 hrs of storage of grass	27.52	1.37	86.80
T ₆ -96 hrs of storage of grass	17.87	0.89	84.90
Mean	18.25	0.91	85.08
SEm±	0.59	0.029	2.33
CD (0.01)	2.08	0.102	NS

Vedhamurthy (2003) reported that, significantly higher oil content and oil yield was observed in 72 hrs of storage of lemongrass in shade (1.37%, 27.52 ml/ 2Kg) followed by 48 hrs of storage of grass (0.93%, 18.62 ml/ 2Kg). This is because as the storage period increased, there is loss of moisture leading to increased cell wall permeability resulting in higher

recovery of oil. But, at 96 hrs of storage, there is decrease in oil recovery because of prolonged storage which resulted in volatilization of the oil along with the loss of moisture beyond the critical limit.

Table 15
Influence of Type of Storage Containers and Duration on
Saponin Content in Safed Musli.

Mishra *et al.*, 2008, Jabalpur (M.P.).

Treatment (Duration)	Saponin content (%)			Mean	% Reduction
	Polythene bags	Plastic contai- ners	Glass contai- ners		
0 months	6.88	6.88	6.88	6.88	-
2 months	6.78	6.82	6.84	6.81	1.01
4 months	6.54	6.65	6.74	6.64	3.48
6 months	6.28	6.46	6.52	6.42	6.68
8 months	6.13	6.10	6.20	6.14	10.75
Mean	6.52	6.59	6.62	-	-
SEm±	0.0221	0.0286	0.0495	-	-
CD @ 5%	0.0638	0.0824	0.1427	-	-

Mishra *et al.*, (2008) reported that, among the three containers (polythene bag, plastic containers and glass containers), storage of powered drug in glass containers retained significantly higher saponin content in all the storage period. The saponin content was reduced significantly from 0 months to 2,4,6,8 month duration by 1.01, 3.48, 6.68, 10.75% respectively and highest reduction being at 8 months after storage. They concluded that the glass containers were found to be the best with least reduction in saponin content.

BIOTECHNOLOGICAL APPROACH

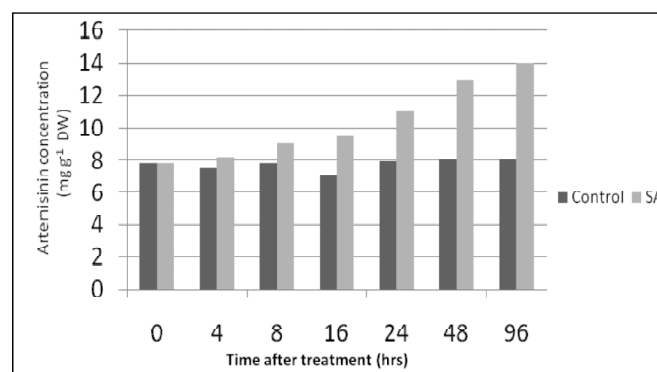


Figure 3: Effect of 1.0 mM SA on the Concentration of Artemisinin in *Artemisia annua* leaves.

Pu *et al.*, 2009, Beijing (China).

Pu *et al.* (2009) reported that, the artemisinin concentration increased slowly during the first 8 hrs after the treatment and a rapid increase was noticed

between 8 hrs and 96 hrs after the treatment recording the highest at 96 hrs after SA (Salicylic Acid) treatment (54 % higher than that of control). This increase is due to the spontaneous conversion of dihydroartemisinic acid (precursor of artemisinin) to artemisinin by reactive oxygen species and by up-regulating the expression of genes involved in artemisinin biosynthesis.

CONCLUSION

Medicinal plants play a vital role for the development of new drugs. Almost, 70% modern medicines in India are derived from natural products. Medicinal plants play a central role not only as traditional medicines but also as trade commodities, meeting the demand of distant markets. India has a very small share (1.6%) of this ever-growing global market.

Stress conditions, results in secondary metabolites accumulation, which play a vital role as defence mechanism in plants. Active principles vary among genotypes which are due to genetic make-up of plants. Seasonal variations alter the Secondary metabolite accumulation. Planting at the right time, fertilizer levels, storage conditions, specific concentrations of plant growth regulators, mutagens are some of the major factors that are influencing the optimum yield of secondary metabolites. Hence, all the factors are to be taken in to consideration before taking up of commercial cultivation of these crops. India has a very small share (1.6%) of this ever-growing global market. To compete with the growing market, there is urgency to expeditiously utilize and scientifically validate more medicinally useful plants.

REFERENCES

- Anita, T. and Agarwal, V. K., (2009), Effect of planting date on yield and essential oil content of rhizomes in sweet flag (*Acorus calamus*). *Biomed.*, **4**(1): 33-35.
- Archna, Harinkheda, D. K. and Mishra, U. S., (2009), Effect of intercropping of *cajanus cajan* on herbage and essential oil yield of *Ocimum basilicum*. *Biomed.*, **4**(2): 113-115.
- Aslam, J., Mujib, A. and Sharma, M. P., (2010), Influence of freezing and non-freezing temperatures on somatic embryogenesis and vinblastine production in *catharanthus roseus* (L.). *Acta Physiol. Plant.*
- Bagchi, G. D., Dwivedi, P. D., Kumar, N., Shukla, R. and Chattopadhyay, S. K., (2009), Effect of growth and season on plumbagin concentration in the roots of *Plumbago zeylanica*. *J. Med. Arom. Pl. Sci.*, **31**: 131-135.
- Chandrashekhara, N., Siddaramappa, R. and Shivananda, T. N., (2006), Effect of fertilizer levels on growth, yield and quality of *Bacopa monnieri*. *Biomed.*, **1**(1): 29-32.
- Hou, J., Li, W., Zheng, Q., Wang, W., Xiao, B. and Xing, D., (2010), Effect of low light intensity on growth and accumulation of secondary metabolites in roots of *Glycyrrhiza uralensis*. *Biochemical Systematics and Ecology.*, **38**: 160-168.
- Kubsad, V. S. and Palled, Y. B., (2009), Effect of date of sowing and stage of harvesting on root yield and quality of ashwagandha under rainfed conditions. *J. Med. Arom. Pl. Sci.*, **31**: 13-16.
- Mamatha, B., Siddaramappa, R. and Shivananda, T. N., (2008), Evaluation of *Mucuna utilis* genotypes for seed and L-DOPA yield. *Biomed.*, **2**(4): 313-318.
- Marchese, J. A., Ferreira, J. F. S., Rehder, V. L. G. and Rodrigues, O., (2010), Water deficit effect on the accumulation of biomass and artemisinin in *Artemisia annua*. *Braz. J. Plant Physiol.*, **22**(1): 1-9.
- Mishra, M., Upadhyay, A. and Dwivedi, S. K., (2008), Influence of type of storage containers and duration on saponin content in Safed musli (*Chlorophytum borivillianum*). *Biomed.*, **3**(2): 142-144.
- Narula, A., Kumar, S. and Srinivastava, P. S., (2005), Effect of abiotic metal stress on diosgenin yield in *Dioscorea bulbifera* L. cultures. *Plant cell Rep.*, **24**: 250-254.
- Pu, G., Ma, D., Chen, J., Ma, L., Wang, H., Li, G., Ye, H. and Liu, B., (2009), Effect of 1.0 mM Salicylic acid on the concentration of artemisinin in *Artemisia annua* leaves. *Plant Cell Rep.* **28**: 1127-1135.
- Ravi, C. S., Sreeramu, B. S. and Mallikarjuna, G. A. P., (2006), Performance of *Solanum nigrum* genotypes for different morphogenetic traits, yield and quality attributes. *Biomed.*, **1**(3): 223-227.
- Shivananda, T. N., Harish, G. U., Rao, V. and Srivastava, H. C., (2009), Effect of different stages of plant growth on recovery of L-DOPA in *Mucuna* spp. *Biomed.*, **4**(2): 137-140.
- Singh, S. K., Salma, K., Shivanand, B. G., Agarwal, A., Harish, G. U. and Shivanand, T. N., (2006), Effect of age of leaf on gymnemic acid content in *Gymnema sylvestre* leaves. *Biomed.*, **1**(2): 159-160.
- Vedhamurthy, T. R., (2003), Post harvest studies in lemongrass varieties (*Cymbopogon flexuosus* Nees). *M. Sc. Thesis*, UAS, Dharwad.

