

Phenology and growth analysis study of ashwagandha [*Withania somnifera* (L.) Dunal.] for yield and alkaloid accumulation under winter sown conditions

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SUMMARY

The experiment was conducted in Herbal Garden, Department of Plant Physiology, Jawaharlal Nehru Agricultural University, Jabalpur during 2002-2003 winter season. The experiment was conducted in a completely randomized design with 10 treatments (various stages of growth and development were the treatments) replicated thrice. The results revealed that *Withania* seeds required 12 days to germinate during winter season. First primary, secondary and tertiary branching were initiated on 20th, 45th and 60th days after sowing (DAS), respectively. There was flower bud initiation after two months (60 DAS). First flowering was within one week after flower bud initiation (65 DAS). Crop took a fortnight to come in 50 per cent flowering (75 DAS). Three months after sowing (90 DAS) first fruit initiation began. Crop required a month (30 days) for 75 per cent fruit set (120 DAS). Fruit maturity period lasted for one and half month thereafter. Crop required ten days for physiological maturity (160 DAS) after 50 per cent fruit maturity and next ten days for physical maturity (170 DAS). The ashwagandha crop has vegetative phase of 65 days and reproductive phase of 70 days. There was significantly higher LAI at first fruit maturity (135 DAS) that was the period at which alkaloid per cent of leaves too was significantly higher. Thus, maximum LAI is the indicator of highest alkaloids in leaves. Relative crop growth (RGR) continued to decrease after 90 DAS. This period onwards alkaloid content was higher in plant part. Crop growth rate which have relevance for rate of dry matter production was indicating negative value at 75 per cent fruit set (120 DAS) and after it. Leaves are the source of alkaloid thus their number, ratio with unit ground (LAI) and biomass were functioning in relation to total alkaloid content in leaves and roots were higher at 135 DAS. Plant height reached to 30.10 cm at maturity which was double than height at 90 DAS (14.31 cm). Root length of crop was one third (10.29 cm) to that of plant height at maturity while alkaloid content was significantly higher at 135 DAS. Leaves/plant were significantly higher in 120 days old crop (60.44 per plant) and there after senescence begins. Similarly, numbers of branches per plant too were significantly higher (5.88/plant) in four months (120 DAS) old crop. The root yield/ha was 6 q/ha while the total biological yield was 11.86 q/ha. The best harvesting time for crop was at 135 to 150 DAS (first fruit to 50% fruit maturity).

Key Words : Ashwagandha, *Withania somnifera*, Alkaloid content

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The experiment was conducted in Herbal Garden, Department of Plant Physiology, Jawaharlal Nehru

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Agricultural University, Jabalpur during 2002-2003 winter season. Ashwagandha [*Withania somnifera* (L.) Dunal] is an important medicinal plants belonging to family Solanaceae, is known as Indian Ginseng in the international market due to aphrodisiac and rejuvenating properties of its roots. All organs of *Withania somnifera* are useful medicinally but roots are commercially the most important one. Roots have neuro- tonic, abortifacient, anti-inflammatory, alterative, aphrodisiac properties and used commonly in consumption, emaciation,

debility due to old age, in hiccup, cough, dropsy, female disorder, ulcers and scabies. Leaves of the plants possess febrifugal properties and applied to carbuncles, lesions, painful swellings and sore eyes. Root and plants are used to cure rheumatic swellings and pain. Seeds have hypnotic properties and also used in coagulating milk. Roots, fruits and seeds are used as diuretic for curing the diseases of urinary tract (Hussain *et al.*, 1992).

The production technology of this crop is well popularized in most of the states of India *viz.*, Madhya Pradesh, Uttar Pradesh, Punjab plain, Himachal Pradesh, Jammu, but practically no work has been taken to study the relationship between physiological growth parameters, morphological growth / yield attributes and root / leaf / alkaloid yield with the fate of alkaloids in different plant parts during crop phenology. Due to this bottleneck in research, normally roots are harvested after seed maturity. There is need to spell out initiation of alkaloid metabolism, its distribution, accumulation in different plant parts when crop is through different growth phases in specific environment. Present investigation is an attempt to ensure maximum alkaloid content in different plant parts based on the morphological and physiological indices for timely crop harvest with the help of biochemical and growth analytical approach.

MATERIALS AND METHODS

The experiment was conducted Herbal Garden, Department of Plant Physiology, Jawaharlal Nehru Agricultural University, Jabalpur during 2002-2003 winter season. The experiment was conducted in a completely randomized design with 10 treatments (various stages of growth and development were the treatments) replicated thrice on variety "Jawahar Aegandh 20" at the rate of 10 kg/ha by hand dibbling seeds at 0.25m row and 0.20m plant to plant distance. Sowing was done in October, 28 and final harvesting was done in April. Total alkaloid content from 45 days after sowing to physiological maturity, its partitioning during different growth and developmental stages in relation to physiological growth characters, phenology and morphological indices of *Withania somnifera* were determined. The treatments were: 30, 45, 60, 75, 90, 105, 120, 135, 150 and 165 Days after sowing. Observations were done at the fixed 15 days interval of growth for leaf area, leaf biomass and shoot biomass and for computation of data for physiological plant growth analytical parameters at successive growth stages (S_1 -30 DAS, S_2 -45 DAS, S_3 -60 DAS, S_4 -75 DAS, S_5 -90 DAS, S_6 -105 DAS, S_7 -120 DAS, S_8 -135 DAS, S_9 -150 DAS and S_{10} -165 DAS). Five plants were randomly selected in each replication, for partitioning into leaf and stem dry matter and measurement of leaf area for growth analysis and alkaloid accumulation. One square meter area was left for obtaining morphological yield attributes of root and seed yield at maturity. Physiological plant growth analytical parameters were computed as formulae given by LAI, LAR LA, SLW,

BMD (McCullum, 1978), RGR (Beadle, 1985), LWR (Beadle, 1952), CGR (Potter and Jones, 1977). The phenology was noted with regard to: Days to germination, first branching, second branching, tertiary branching, days to floral primordial initiation, days to floral initiation, days to 50 per cent flowering, days to first fruit initiation, days to 50 per cent fruiting, days to first fruit maturation, days to 50 per cent fruit maturity and days to physiological maturity. Alkaloid analysis of partitioned dried stem, leaf and root of *Withania somnifera* was done at different stages of growth and development (treatments) as per procedure for total alkaloid analysis (Majumdar, 1962). Analysis of different variables was carried out to know the degree of variation amongst all the treatments. The data collected at different growth stages were analyzed for completely randomized design by the method given by Fisher (1967).

RESULTS AND DISCUSSION

The results of the present study as well as relevant discussion have been presented under following heads:

Phenology :

Phenology of ashwagandha (*Withania somnifera*) when investigated in winter season (sown on 28th October) in vertisol soils it took 12 days to germinate. Branch initiation began 20 days after sowing while second branch was initiated within a week after initiation of first branch. Crop took more than 30 days for tertiary branch initiation. There was flower bud initiation after two months (60 DAS). First flowering was seen within one week after flower bud initiation (65 DAS). It took a fortnight for the crop to come in 50 per cent flowering (75 DAS). Flowering period was of 20 days. Three months after sowing (90 DAS) first fruit initiation began. Crop required a month for 75 per cent fruit set (120 DAS). Fruit maturity period lasts for one and half months (150 DAS). Crop required ten days for physiological maturity (160 DAS) after 75 per cent fruit set and next ten days for physical maturity (165 DAS). The ashwagandha crop has vegetative phase of 65 days and reproductive phase of 70 days (Table 1).

Physiological yield attributes :

Functional attributes of productivity investigated through growth analysis parameters were expressed differently by the crop in total crop period (Table 2 and 3). There was no significant variation in LAI up to 105 DAS. There was significantly higher LAI at first fruit maturity (135 DAS). That was the period at which alkaloid content in leaves too was significantly higher. Thus, maximum LAI is the indicator of highest alkaloids in leaves. Leaf Area Index was significantly higher (2.662) at 135 DAS. BMD were also significantly higher. However, LAD and per cent alkaloid were higher at 120 DAS (75 per cent fruiting stage). There was apparently no pattern of thickness of leaf (SLW) and leafiness (SLA) during crop

Table 1 : Alkaloid content (%) modeling

	Root alkaloid content (%) models:	Leaf alkaloid yield (%) models	Stem alkaloid yield (%) models-
1.	$Y_k=0.1941 + 0.00086 \times \text{LAI}$	$Y_j=0.2168 + 0.00448 \times \text{LAI}$	$Y_k=0.1941 + 0.00086 \times \text{LAI}$
2.	$Y_i=0.1856 + 0.00008 \times \text{LDW}$	$Y_j= 0.2180 + 0.00006 \times \text{LDW}$	$Y_k=0.1941 + 0.00086 \times \text{LAI}$
3.	$Y_i=0.1883 + 0.00002 \times \text{TDM}$	$Y_j=0.2175 + 0.00002 \times \text{TDM}$	$Y_k=0.1939 + 0.00001 \times \text{LDW}$
4.	$Y_i= 0.2342 + 4.19821 \times \text{LAR}$	$Y_j =0.1760 + 3.83237 \times \text{LAR}$	$Y_k=0.1944 + 0.00000 \times \text{TDM}$
5.	$Y_i=0.2540 + 0.09401 \times \text{LWR}$	$Y_j=0.1673 + 0.06820 \times \text{LWR}$	$Y_k=0.2016 + 0.68917 \times \text{LAR}$
6.	$Y_i=0.2194 + 1.17799 \times \text{SLA}$	$Y_j=0.1772 + 1.98744 \times \text{SLA}$	$Y_k=0.2043 + 0.01442 \times \text{LWR}$
7.	$Y_i=0.2005 + 0.00004 \times \text{SLW}$	$Y_j=0.2177 + 0.00016 \times \text{SLW}$	$Y_k=0.1999 + 0.24734 \times \text{SLA}$
8.	$Y_i = 0.1762 + 0.00060 \times \text{LAD}$	$Y_j=0.2213 + 0.00038 \times \text{LAD}$	$Y_k=0.1961 + 0.00001 \times \text{SLW}$
9.	$Y_i=0.1792 + 0.00000 \times \text{BMD}$	$Y_j=0.220 + 0.00000 \times \text{BMD}$	$Y_k=0.1926 + 0.00000 \times \text{BMD}$
10.	$Y_i=0.2019 + 0.00016 \times \text{CGR}$	$Y_j=0.2035 + .00003 \times \text{CGR}$	$Y_k=0.1960 + 0.00005 \times \text{CGR}$
11.	$Y_i=0.2069 + 0.08452 \times \text{RGR}$	$Y_j=0.1958 + 0.20887 \times \text{RGR}$	$Y_k=0.1969 + 0.00821 \times \text{RGR}$
12.	$Y_i=0.2035 + 0.00002 \times \text{NAR}$	$Y_j=0.2007 + .00033 \times \text{NAR}$	$Y_k=0.1925 + 0.00001 \times \text{NAR}$

Where Y_i = Root alkaloid yield variable, Y_j = leaf alkaloid yield variable, Y_k = Stem alkaloid yield (%)

Table 2: Morphological attributes of root and seed yield at different stages of growth in *Withania somnifera* and alkaloid content (%) in different plant parts at different growth

Sr. No.	Stages of plant growth	Plant height (cm)	Root length (cm)	No. of leaves /plant	No. of stem, branches /plant	Root dry weight (kg/ha)	Stem dry weight (kg/ha)	Leaf dry weight (kg/ha)	Alkaloid content (%)		
									Leaf	Stem	Root
1.	30 DAS	2.79	1.805	2.33	0.00	0.15	0.03	15.00	0.000	0.0000	0.0000
2.	45 DAS	3.87	3.10	5.00	0.40	25.00	4.00	23.00	0.2407	0.1900	0.1803
3.	60 DAS	7.10	3.826	6.22	1.00	28.00	38.00	97.00	0.2017	0.1913	0.1817
4.	75 DAS	11.97	5.676	7.776	2.50	44.00	300.00	111.00	0.227	0.2000	0.2190
5.	90 DAS	14.31	5.976	25.55	2.66	256.00	318.00	398.00	0.1917	0.2020	0.2273
6.	105 DAS	14.88	8.193	47.55	4.78	300.00	570.00	455.00	0.1943	0.2053	0.2413
7.	120 DAS	15.83	8.213	60.44	5.89	494.00	772.00	706.00	0.1837	0.1973	0.2230
8.	135 DAS	29.33	9.22	53.22	5.33	565.00	890.00	358.00	0.1803	0.1930	0.2083
9.	150 DAS	29.88	9.553	46.55	5.22	585.00	946.00	309.00	0.1813	0.1930	0.2083
10.	165 DAS	30.10	10.29			596.00	998.00	90.00	0.1780	0.1840	0.1997
	S.E. ±	7.8	1.72	6.86	0.34	7.55	40.20	39.40	0.0016	0.0011	0.0015
	C.D. (P=0.05)	16.26	3.59	14.41	0.71	21.02	110.02	105.00	0.0047	0.0033	0.0044

Table 3: Leaf area index (LAI), leaf weight ratio (LWR), leaf area duration (LAD), biomass duration (BMD), LAR, SLW, SLA, RGR, CGR and NAR in (*Withania somnifera*) at different growth stages

Sr. No.	Stages of plant growth	LAI	LWR	LAD	BMD	LAR	SLW	SLA	RGR g/g/day	CGR g/m ² /day	NAR g/m ² /day
1.	45 DAS	0.065	0.35	1.47	103.23	0.0053	21.964	0.0051	0.0167	0.3386	0.040
2.	60 DAS	0.131	0.24	2.53	387.46	0.0051	38.426	0.0070	0.0348	0.2549	1.2294
3.	75 DAS	0.205	0.23	3.76	2558.07	0.021	51.620	0.0050	0.0015	2.1878	9.518
4.	90 DAS	0.296	0.18	9.94	1453.05	0.0016	41.991	0.0048	0.0421	3.210	16.051
5.	105 DAS	1.030	0.14	20.88	4786.39	0.0016	28.415	0.0044	0.0018	16.596	3.126
6.	120 DAS	1.754	0.11	33.12	7406.45	0.0015	26.478	0.0042	0.0134	20.870	0.217
7.	135 DAS	2.662	0.11	31.37	8553.46	0.0014	22.415	0.0038	-0.0043	-9.8831	-2.036
8.	150 DAS	1.520	0.07	18.53	6951.36	0.0010	22.403	0.0037	-0.0286	-4.357	-3.029
9.	165 DAS	0.950	0.07	0.814	895.69	0.0007	22.234	0.0035	0.0064		
	S.E. ±	0.341	0.01	3.42	968.20	0.0053	4.3392	0.0052	0.0194	7.2206	3.126
	C.D. (P=0.05)	1.014	0.05	10.26	292.68	0.00103	12.892	0.00156			9.372

growth period. There was no direct significant correlation of these two parameters with root, leaves and stem alkaloid. Relative crop growth (RGR) continued to decrease after 90 DAS. This period onwards alkaloid content was higher in plant part. This indicates that respiratory losses were more than relative dry matter accumulated. This might be due to the fact that alkaloid synthesis might have metabolized more raw (sugar) material to metabolize alkaloids. This resulted in decrease in relative crop growth rate rather increase, as it happens in other crops. Crop growth rate which have relevance for rate of dry matter production was indicating negative value and 75 per cent fruit (120 DAS) and after it. This supports earlier statement that more energy was consumed by crop in alkaloid synthesis than for crop dry weight increase. Leaves are the source of alkaloid thus their number, ratio with unit ground (LAI) and biomass were functioning in relation to total alkaloid content in leaves (productivity) and roots, respectively were higher at 135 DAS.

Morphological yield attributes :

Physiological processes resulted in expression of structural attributes of productivity in terms of increase in stem and root length, number and weight of plant parts were quantified at 15 days interval from sowing to physical maturity (Table 2). Analysis revealed the following facts: Plant height reached to 30.10 cm at maturity which was double than height at 90 DAS (14.31 cm). Root length of crop was one third (10.29 cm) to that of plant height at maturity. Roots being the major source of alkaloid they did have less growth in terms of length as well as root dry weight. Thus, it is worth harvest crop in between 135 to 150 at which crop has first fruit to 50 per cent fruit maturity. Leaves per plant were significantly higher in 120 days old crop (60.44 per plant) and there after senescence begins. Similarly number of branches per plant too was significantly higher (5.88/plant) in four months (120 DAS) old crop.

Productivity or yield and alkaloid accumulation:

Productivity or yield per hectare is the marketable produce or economically important end product of physiological processes. In case of ashwagandha it is root that too has alkaloid. The root yield/ha was 5.85 q/ha while the total biological yield was 18.40q/ha. Alkaloid accumulated in plant parts were analyzed at 15 days intervals with effect from 45 days after sowing to maturity. Pachori (1995) had analyzed total alkaloid content of plant parts and reported variation in alkaloid content with age. Leaves continued to synthesize and accumulate more alkaloid up to 75 DAS while distribution to stem and roots was there with concomitant increase up to 105 and 120 days after sowing, respectively. Maximum alkaloid per cent was at 75 DAS, 105 DAS and 120 DAS in leaves stem and roots, respectively. Beyond 120 days leaves, stem and roots had significant reduction in alkaloid

per cent. Plant parts have significantly lowest alkaloid per cent at maturity *i.e.* 0.1780, 0.1840 and 0.997 per cent, respectively in leaves, stem and roots. Evans (1989) and Verma (2002) had discussed the distribution of alkaloid from the place of biosynthesis to other plant parts. However, they did not signify the growth stages of plant species. Stem might have acted as carrier of alkaloid from leaves to roots *i.e.* why its alkaloid productivity was meager as compared to leaves and roots. A meager amount is left in stem while its translocation towards roots. The accumulation of alkaloids in plant parts other than where synthesized was also suggested by Verma (2002) and Pachori (1995). Hence, the above results of alkaloid partitioning are in conformity with the results of above scientists. Alkaloid per cent remained approximately constant in between 135 to 150 days in almost all parts *i.e.* leaf, stem and root and reaching to 0.1780, 0.1840 and 0.1997 per cent, respectively at maturity. The study was first of its type that critically advise the morphological indices and phenology at which the crop should be harvested. If ashwagandha crop is harvested at physical maturity there was loss in alkaloid per cent in roots reaching to 0.1997 per cent while it was 0.2083 per cent from 135 DAS to 150 DAS. It is against the common practice of harvesting any crop at physical maturity. Similar practice to harvest ashwagandha roots at physical maturity as proposed by Trivedi *et al.* (1976), Maheshwari (2000), of the crop may lead to alkaloid losses.

Alkaloid content (%) modeling :

Partitioning of alkaloid (%) in different plant parts *viz.*, root, leaf and stem at any stage of growth /development were modeled taking individual physiological yield attributes, leaf /plant biomass productivity as a variable using single factor regression equation. These models will be very useful in predicting alkaloid partitioning in winter sown *Withania somnifera*.

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