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Changes in environmental conditions influence the growth, yield, and quality attributes of ashwagandha (*Withania somnifera*) cultivars

Anjali Singh[1,2], Saudan Singh[1], Anil Kumar Singh[1] and Rajesh Kumar Verma[2]*

[1]Crop Production and Protection Division, CSIR-Central Institute of Medicinal and Aromatic Plants, Lucknow 226015, Uttar Pradesh, India

[2]Academy of Scientific and Innovative Research (AcSIR), Ghaziabad 201002, India

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Ashwagandha (*Withania somnifera* L. Dunal) is an important medicinal plant belonging to the family Solanaceae with significant pharmacological and economic value due to its increasing demand in nutraceutical and pharmaceutical industries. A field experiment was conducted at the research farm of CSIR-Central Institute of Medicinal and Aromatic Plants (CSIR-CIMAP), Lucknow, for two consecutive years (2021–22 and 2022–23) to optimise plant population and harvesting stages of two cultivars (NMITLI-118 and NMITLI-101) under summer and winter seasons for maximising yield and quality. The results showed significant effects of cultivars (C), plant population (P), harvesting stage (H), and their interactions (C × P × H). The treatment combination C1 × P2 × H2 recorded maximum dry leaf (2.04 t/ha), root (1.74 t/ha), and seed yields (0.67 t/ha). The summer-grown crop harvested at full flowering stage (120 DAS) exhibited superior growth, photosynthetic activity, and quality traits, including withanolide content (0.68%), starch (23.7%), and crude fibre (41.22%). Higher economic returns were obtained with 20 × 15 cm spacing in cv. NMITLI-118 during summer. Overall, optimised agronomic practices and suitable cultivars significantly enhance the yield, quality, and profitability of ashwagandha cultivation. Adopting suitable ashwagandha cultivars and optimised agronomic practices can enhance yield and quality, thereby meeting pharmaceutical demand and improving farmers' incomes.

Keywords: Ashwagandha, Cultivars, Growth attributes, Harvest stage, Plant density, Seasonal variation, Withanolides, Yield

IPC code; Int. cl. (2021.01)– A01G

Introduction

Withania somnifera (L.) Dunal, commonly known as ashwagandha, is an important medicinal plant belonging to the family Solanaceae. It is widely used in traditional Indian medicine due to the presence of steroidal lactones known as withanolides, including withaferin A, withanolide A, withanolide D, and withanone[1-3]. These bioactive compounds exhibit diverse pharmacological properties such as immunomodulatory, cardioprotective, neuroprotective, anti-inflammatory, anti-tumour, anti-stress, and antioxidant activities[4,5].

Distribution and economic importance

Ashwagandha is widely distributed in the drier subtropical regions of India and is also cultivated in several countries, including Pakistan, Afghanistan, Egypt, and South Africa[6]. In India, major cultivation areas include Madhya Pradesh, Uttar Pradesh, Rajasthan, Gujarat, Haryana, and Punjab[4,5,7]. The crop has gained considerable economic importance due to its increasing global demand, particularly in the nutraceutical and pharmaceutical industries. The global market for ashwagandha is expanding rapidly, driven by increasing demand for herbal and natural products[8]. India is a major producer and exporter of ashwagandha roots. In recent years, both cultivated area and production have increased significantly; however, the demand still exceeds supply. The global market value of ashwagandha extract is projected to grow substantially in the coming years, highlighting the need to improve both yield and quality. The worldwide market for ashwagandha extract was assessed at $864.3 million in 2021 and is anticipated to attain a valuation of $ 2.5 billion by 2031, reflecting a compound annual growth rate of 11.4% from 2022 to 2031[(Ref. 9)].

Influence of environmental factors

Despite its economic potential, variability in cultivation practices, environmental conditions, and cultivar selection often results in inconsistent quality

*Correspondent author
Email: rajesh.verma@cimap.res.in, rajeshcimap@rediffmail.com

of produce. Plants interact with the environment as they grow and develop, coming into contact with various abiotic elements such as light, water, temperature, soil, and chemicals[10,11]. The plant has a slow growth rate, a relatively low concentration of these bioactive substances, and an accumulation pattern that is highly sensitive to environmental and geographic factors[11,12]. Therefore, varietal adaptability to the environment and fluctuations is also crucial for stabilising crop production across regions and years.

Role of agronomic factors

In ashwagandha, the accumulation of bioactive compounds is highly sensitive to these environmental variables.

In addition, agronomic factors such as plant population density (spacing), growing season, and harvesting stage significantly affect growth, yield, and quality traits. Optimal plant density ensures efficient resource use, while an appropriate harvesting stage is critical for maximising secondary metabolite accumulation, particularly withanolide. Although several studies have investigated individual agronomic factors, comprehensive studies evaluating the combined effects of cultivar, plant population, growing season, and harvesting stage are limited. Moreover, genotype × environment interactions in ashwagandha remain insufficiently explored. The lack of standardised agronomic practices and suitable high-yielding cultivars for different agro-climatic conditions continues to limit productivity.

Objectives of the study

Therefore, there is a need to identify optimal combinations of these factors to enhance yield, quality, and economic returns. The present study was undertaken with the following objectives: (i) To evaluate the effects of growing seasons, cultivars, plant population, and harvesting stages on growth and photosynthetic parameters; (ii) To assess root yield and quality attributes, including withanolide content; (iii) To analyse the economic feasibility of ashwagandha cultivation under different treatment combinations.

Materials and Methods

Experimental site

The field experiment was conducted for two consecutive years (2021–22 and 2022–23; October to May) at the experimental farm of CSIR–Central Institute of Medicinal and Aromatic Plants (CSIR-CIMAP), Lucknow, India (Fig. 1). The site is located at 26.5° N latitude and 80.5° E longitude, at an altitude of 120 m above mean sea level. The region falls under a semi-arid subtropical climate, characterised by hot summers and moderately cool winters.

The monsoon season typically extends from the last week of June to September, with approximately 80% of the annual rainfall occurring during July–August. Meteorological data, including temperature, relative humidity, and rainfall during the experimental period, are presented in Fig. 2. The soil of the experimental field was sandy loam, with a pH of 8.0 and an electrical conductivity (EC) of 3.44 dS/m.

Procurement of seeds

The quality seeds of *cv.* NMITLI-118 and NMITLI-101 were procured from the experimental farm of CSIR-CIMAP, Lucknow, from the previous year's mother plants. The major characteristics of the selected cultivar were tested during the present investigation, including:

NMITLI-118: The first pharmacologically validated Ashwagandha variety was jointly developed

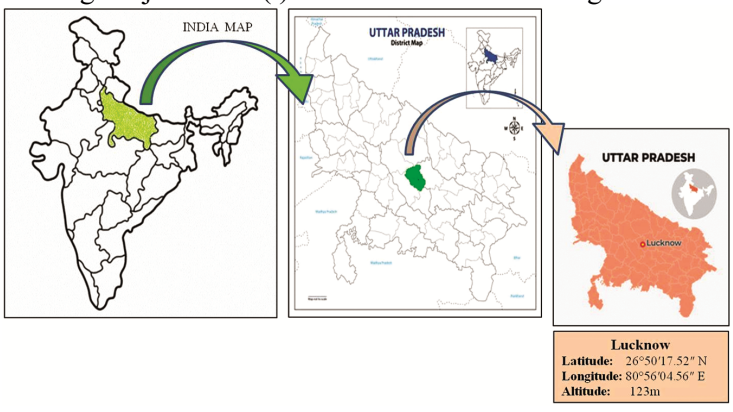

Fig. 1 — Map Location of the experimental site during both the experimental period (summer and Winter Season).

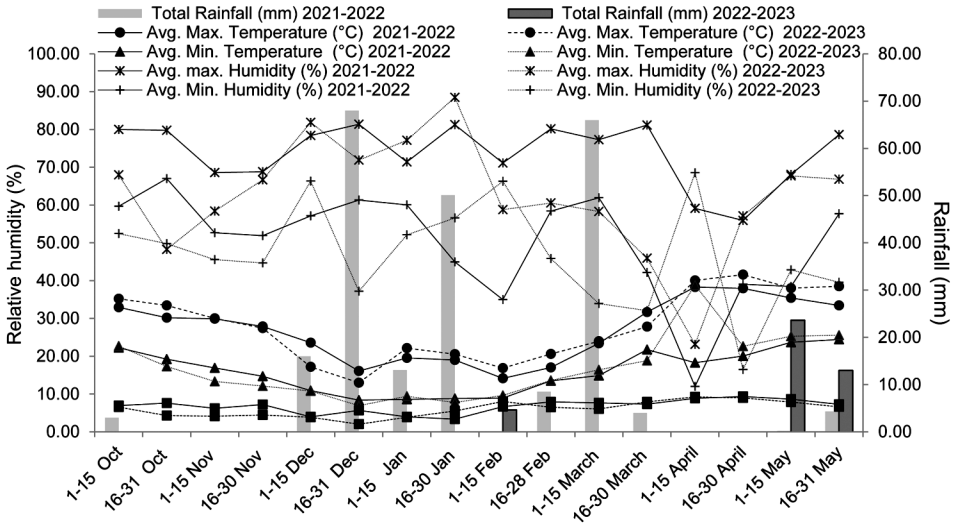

Fig. 2 — Mean of standard fortnight air temperature (°C), relative humidity (%), and total rainfall (mm) over the cropping period (October 2021–May 2023).

by CSIR-CIMAP, Lucknow, and CSIR-National Botanical Research Institute (NBRI), Lucknow, in 2009. This phytochemical uniform variety includes a uniform crop canopy, more plants per unit area due to non-spreading plant architecture, and high dry root yield and high withanolide yield per unit biomass. The roots contain withanolide A and withanone, and the leaves have up to 2% withaferin A with no withanone. It has an indeterminate type of growth habit and triparous branching. It is non-lodging and drought-resistant. The branches are covered with stellate hairs, appearing tomentose to sparsely glabrous along their length. The leaves are simple and arranged oppositely. The plants are bushy, typically 80–100 cm tall. Flowers are inconspicuous, white to dull yellow in colour, sessile, axillary, and bisexual. The plant is medium in maturity. Berries are yellow in colour. Roots are smooth, starchy, and brownish in colour.

NMITLI-101: This high-yielding, improved immunomodulatory variety was released by CSIR-CIMAP, Lucknow, in 2015. Under optimal agroclimatic conditions, the variety can yield high dry root yields and total withanolide content. Characteristics morphological features include dark green leaves of medium size, medium height, profuse branching, red-colored berries, etc. The NIMITLI variety was introduced and commercialised in Tikampur district, Madhya Pradesh, by the National Bank for Agricultural and Rural Development. It is non-lodging and drought-resistant. The branches are covered with stellate hairs, appearing tomentose, and become somewhat glabrous with age. Leaves are ovate and opposite, dark green. Plants are bushy, typically 70-90 cm tall. Flowers are inconspicuous, white to dull yellow in colour, sessile axillary, umbellate cyme, and bisexual. It is medium in maturity. Berries are yellow in colour. Roots are smooth and starchy, having a brownish colour.

Experimental and treatment details

The experiment was laid out in a factorial randomised block design with three replicates, each with an individual plot size of 6.0 m^2 (3.0 m × 2.0 m). Before sowing through the top dressing, the recommended basal doses of N (45 kg/ha), P_2O_5 (25 kg/ha), and K_2O (20 kg/ha) were applied in the forms of urea, single super phosphate (SSP), and muriate of potash (MOP). Seed sowing was done, and plant spacing (population density) was maintained according to experimental need. The half remaining dose of N (20 kg ha^{-1}) was applied at the first 50 days of sowing, and the rest of N at 90 days after sowing. After 25 days of sowing. All intercultural operations were carried out manually as needed. Harvesting was done as per the treatment, and the attribute-related data were collected and compiled.

C_1: *cv.* NMITLI-118, and C_2: *cv.* NMITLI- 101; four plant populations, i.e., P_1: 2,50,000 plants/ha (20×20 cm spacing), P_2: 3,33,333 plants/ha (20×15 cm spacing), P_3: 5,00,000 plants/ha (20×10 cm spacing), and P_4:10,00,000 plants/ha (20×05 cm spacing); and three harvesting stages, i.e., H_1: pre-flowering stage (90 DAS), H_2: full flowering stage (120 DAS), and H_3: seed maturity stage

(180 DAS), and two different seasons i.e., S_1: winter season and S_2: summer season (S_2) (Table 1.)

Biometric observations

Growth observations, including plant height, canopy spread, number of branches per plant, number of leaves per plant, root length, and leaf area index (LAI), were recorded at different stages of harvesting for each treatment. Five plants were selected randomly, and the average data were subjected to statistical analysis. Plant height was measured with a meter scale from the base of the plant to the tip of the shoot, and the number of branches and leaves per plant was counted manually. Root length was measured using a physical measuring method with a ruler. LI-COR's LAI 2200C analyser was used to measure leaf area index (LAI). The Dual-PAM-100 was used to measure chlorophyll fluorescence (Heinz Walz GmbH, model: Dual-PAM-100).

Harvesting and yield observations

Harvesting was conducted according to the treatment, and the total weight of each plot was recorded. The weight of sampled plants was added to the herbage yield of the respective treatments. Then, the herb was left to dry in the shade for the next 10-15 days to obtain a constant dry weight of leaves, stems, and berries. The dry weight of the total biomass, roots, and berries of the harvested plants was measured and expressed on a t ha^{-1} basis for comparative yield measurement.

Quality analysis

Sample preparations

The roots were completely dried in shade conditions for their quantitative and qualitative evaluation by the method[13]. Dried roots were cut into small pieces and then made into a powder. A 50 mg powder was weighed accurately in duplicate and transferred into a 15 mL capacity glass tube. Rectified ethanol (4 mL) was added to the tube containing the root part. The tubes were heated for 1 h on a water bath maintained at 50°C. After 1 h, the tubes were removed from the water bath and allowed to cool down to room temperature. The supernatant was transferred to another tube. The residue left in the tube was subsequently re-extracted twice with ethanol (2 mL each) by centrifugation. The solvent was completely evaporated from the samples in a rotary evaporator, then dissolved in 1 mL of HPLC-grade methanol (Merck, India). Prepared Samples were filtered through Swinnex polypropylene 25-mm filter holders (Millipore, USA) containing Durapore 0.22-micron filters, placed in 1 mL HPLC vials, and centrifuged at 10,000 rpm at room temperature for 3-5 min. These samples were then subjected to HPLC analysis.

High-performance liquid chromatography (HPLC) analysis

It was then filtered and evaporated using a rotary evaporator. N-hexane (10 mL × 3) was used to make the extract fat-free, and the extract was then treated with 50 mL of 1% H_2SO_4. After that, with the help of diethyl ether (30 mL×3), the defatted solution was extracted in a separating funnel. The upper layer (ether-soluble) was used for the determination of withanolides. The lower layer (acid-soluble) was used for total alkaloid estimation, and further processing was performed as described by Khajuria *et al.*[14]. Using a water spheris or C18 analytical column (4.6 mm × 250 mm, 10 mm ODS), reverse phase HPLC was used to remove withanolides A and total withanolides content from samples at the CSIR-CIMAP analytical facility. To calculate the yield of respective chemical components in kg/ha, withanolides a (%) and total withanolides (%) were multiplied by dry root yield[15].

Chlorophyll and carotenoids content analysis

The total chlorophyll and carotenoids are extracted in 80% acetone as the method given by Arnon[16], and absorption was taken at wavelengths 663, 645, 480, and 510 nm, respectively, in a UV spectrophotometer as follows:

Table 1 — Experiment details of the treatments during two different growing seasons (winter and summer)

Experimental details Season: Summer and Winter			
Cultivars	Plant Populations (Plants/ha)	Total plot Area (m^2)	Harvesting Stages
NMITLI-118	2,50,000 (20×20cm)	700.80m^2	Pre-flowering (90DAS)
	3,33,333 (20×15cm)		Full flowering (120DAS)
NMITLI-101	5,00,000 (20×10cm)		Seed Maturity (180DAS)
	10,00,000 (20×05cm)		

[a]Total cultivar = 02, Total plant population = 04; Total harvesting stages = 03 (2×4×3 = 24 treatment combinations), the number of replications (i.e., 03) in two different seasons i.e., Summer and Winter.

(1) mg chlorophyll a/g tissue= 12.7 (A663-2.69 (A645) × v/1000×w

(2) mg chlorophyll b/g tissue= 22.9 (A645-4.68 (A663) × v/1000×w

(3) mg total chlorophyll/g tissue= 20.2 (A645+8.02 (A643) × v/1000×w

(4) mg carotenoids/g tissue= 7.6 (A+80) -1.49 (A510) × v/1000×w

where, A = Absorbance at a specific wavelength, V = Final volume of chlorophyll extract in 80% acetone, and W = Fresh weight of tissue extracted.

Chemical analysis

For chemical analysis, the dried root material was ground in a pulveriser, sieved, and the fine root powder was used for the analysis. Root starch content was determined[17] and crude fibre content was quantified[18]. Starch–fibre ratio was calculated as under:

$$\left[\text{Starch: Fibre ratio(SFR)} = \frac{\text{Starch content \%}}{\text{Fibre content \%}}\right]$$

Economics

Total cost of cultivation (TCC)

The present investigation was evaluated economically using gross returns, net returns, and the benefit-cost ratio. The total cost of cultivation (TCC) of ashwagandha was calculated based on the input costs of various operations and materials used, such as seeds, fertilisers, intercultural practices, and crop management. The cost of essential inputs and outputs was based on the relevant year's standard market price.

Gross returns (GR)

The financial recovery of the essential oil extracted from the ashwagandha crop was multiplied by the average market price to estimate the crop's gross returns. A U.S. dollar was used to calculate gross profits. The standard market price was determined by APEDA (price was determined at average market rates during 2021-2023 by APEDA). Gross returns were calculated using the following formula:

Gross return (GR) = Essential oil yield × Respective market price

Net returns (NR)

The term "net returns" refers to the actual money made by farmers. By deducting the total cost of cultivation (TCC), which includes labour and materials, from the crop's gross returns, the crop's net returns were determined. U.S. Dollars were used to calculate net profits. The equation for calculating total profits is as follows:

Net return (NR) = Gross return (GR) × Total cost of cultivation (TCC)

Benefit-cost ratio (B: C ratio)

Throughout the growing season, farmers' financial returns on their investments are measured by the benefit-cost ratio. The ratio of the total gross returns to the total cultivation costs is used to determine profitability. To calculate the B: C ratio, use the formula below:

$$\left[\text{Benefit: Cost Ratio} = \frac{\text{Net Return}}{\text{Total Cost of Cultivation}}\right]$$

Statistical analysis

The recorded data from both experimental years were statistically examined using a three-factorial design in a Factorial Randomised Block Design (FRBD)[19]. Means within each treatment group were compared using critical difference (CD) values calculated at the 5% significance level ($P \leq 0.05$). The cultivars, plant population, and harvesting stages were treated as fixed effects, and different planting seasons were treated as random effects in the analysis. The sources of variation in an individual-based analysis of variance were classified as blocks, treatments, and error terms. In a combined analysis of variance, the treatment effect was divided into four components: C×P, C×H, P×H, and C×P×H effects.

Results and Discussion

Growth attributes

The data presented in Tables 2–4 indicate that growth parameters of *Withania somnifera* were significantly influenced by growing season, plant population, and harvesting stage across both cultivars. Among the seasons, the summer season (S2) recorded significantly higher values for plant height
(130.47 cm), number of branches (21.06/plant), number of leaves (271.18/plant), canopy spread (45.53 cm), root length (19.63 cm), root girth (19.09 mm), and leaf area index (LAI; 5.46) in cultivar C_1. This enhanced growth during summer may be attributed to favourable environmental conditions, such as higher temperatures, longer photoperiods, and increased light intensity, which promote photosynthetic activity and biomass accumulation.

Plant population also significantly affected growth attributes. An increase in plant density led to higher plant height and LAI, likely due to competition for light, which stimulates vertical growth. However,

Table 2 — Effects of different cultivars, plant populations, and stages of harvest on plant height and numbers of branches/plant, in *Withania somnifera* (mean values of the two years' data)

Cropping period Treatments	Plant height (cm)		Number of branches/plant	
	Winter season (S_1)	Summer season (S_2)	Winter season (S_1)	Summer season (S_2)
		Cultivars		
(C_1)	102.66	130.47	16.39	21.06
(C_2)	90.69	109.22	10.00	19.25
CD at 5%	4.98	6.21	0.71	1.23
		Plant spacing (cm)		
(20×20cm) (P_1)	90.35	109.48	13.63	21.44
(20×15cm) (P_2)	96.65	120.81	13.57	20.75
(20×10cm) (P_3)	98.89	123.61	13.37	20.44
(20×05cm) (P_4)	100.39	125.49	12.21	18.00
CD at 5%	7.04	6.21	1.01	1.74
		Harvesting stages (DAS)		
(90DAS) (H_1)	93.80	116.41	12.07	16.87
(120DAS) (H_2)	95.96	120.27	12.59	20.41
(180 DAS) (H_3)	98.99	122.87	14.93	23.18
CD at 5%	6.10	07.61	0.87	1.50
		Interactions		
	CD at 5%	CD at 5%	CD at 5%	CD at 5%
C×P	9.98	12.42	1.43	2.46
C×H	8.64	10.76	0.90	2.13
P×H	12.22	15.21	1.75	3.01
C×P×H	17.29	21.51	2.50	4.25

[a] Level of significance ($P \leq 0.05$)

[b] C_1: NMITLI-118, C_2: NMITLI-101, P_1: 2, 50,000 plants ha^{-1}, P_2: 3, 33,333 plants ha^{-1}, P_3: 5, 00,000 plants ha^{-1}, P_4: 10, 00,000 plants ha^{-1}, H_1: Pre- flowering harvest stage, H_2: full flowering harvest stage, H_3: seed maturity harvest stage. DAS: Days after sowing

Table 3 — Effects of different cultivars, plant populations and stages of harvest on numbers of leaves plant/leaves canopy spread (cm^2), and leaf area index (LAI) in *Withania somnifera* (mean values of the two years data).

Cropping period Treatments	Numbers of leaves/plant		Canopy spread (cm^2)		Leaf area index (LAI)	
	Winter season (S_1)	Summer season (S_2)	Winter season (S_1)	Summer season (S_2)	Winter season (S_1)	Summer season (S_2)
			Cultivars			
(C_1)	221.30	271.18	34.37	45.53	4.6	5.46
(C_2)	208.84	255.53	23.02	25.59	4.46	5.24
CD at 5%	11.10	13.55	1.52	2.31	0.23	0.32
			Plant spacing (cm)			
(20×20cm) (P_1)	223.87	273.45	29.60	36.76	4.20	4.96
(20×15cm) (P_2)	220.51	269.34	29.51	36.56	4.59	5.43
(20×10cm) (P_3)	216.54	264.54	29.12	36.01	4.66	5.50
(20×05cm) (P_4)	201.50	246.10	26.46	32.90	4.68	5.51
CD at 5%	15.70	19.17	2.16	3.27	0.33	0.45
			Harvesting stages (DAS)			
(90DAS) (H_1)	213.82	252.84	26.46	32.33	3.94	4.71
(120DAS) (H_2)	227.80	276.76	28.07	40.07	4.87	5.76
(180 DAS) (H_3)	205.19	260.46	31.56	34.28	4.78	5.58
CD at 5%	13.60	16.60	1.87	2.83	0.29	0.39
			Interactions			
	CD at 5%	CD at 5%	CD at 5%	CD at 5%	CD at 5%	CD at 5%
C×P	22.12	13.55	3.05	4.62	0.46	0.64
C×H	19.20	23.48	3.01	4.00	0.30	0.55
P×H	27.10	33.20	3.73	5.66	0.55	0.78
C×P×H	38.31	46.95	5.30	8.00	0.80	1.10

[a] Level of significance ($P \leq 0.05$)

[b] C_1: NMITLI-118, C_2: NMITLI-101, P_1: 2, 50,000 plants ha^{-1}, P_2: 3, 33,333 plants ha^{-1}, P_3: 5, 00,000 plants ha^{-1}, P_4: 10, 00,000 plants ha^{-1}, H_1: Pre- flowering harvest stage, H_2: full flowering harvest stage, H_3: seed maturity harvest stage. DAS: Days after sowing

Table 4 — Effects of different cultivars, plant populations and stages of harvest on root length (cm), and root girth (mm) in *Withania somnifera* (mean values of the two years data)

Cropping period Treatments	Root length (cm)		Root girth (mm)	
	Winter season (S_1)	Summer season (S_2)	Winter season (S_1)	Summer season (S_2)
		Cultivars		
(C_1)	14.63	19.63	16.0	19.09
(C_2)	10.19	12.80	8.50	11.93
CD at 5%	0.66	1.00	0.90	1.00
		Plant spacing (cm)		
(20×20cm) (P_1)	13.08	15.36	11.0	16.9
(20×15cm) (P_2)	12.77	16.37	12.3	15.6
(20×10cm) (P_3)	12.67	16.43	12.5	15.4
(20×05cm) (P_4)	11.13	16.68	12.9	14.1
CD at 5%	0.93	1.42	1.00	1.40
		Harvesting stages (DAS)		
(90DAS) (H_1)	10.46	13.87	9.8	11.0
(120DAS) (H_2)	12.07	15.94	13.8	16.7
(180 DAS) (H_3)	14.71	18.83	13.0	18.9
CD at 5%	0.90	1.23	1.10	1.20
		Interactions		
	CD at 5%	CD at 5%	CD at 5%	CD at 5%
C×P	1.31	2.01	1.4	2.0
C×H	1.14	1.23	1.2	1.7
P×H	1.61	1.74	1.7	2.5
C×P×H	2.30	3.48	2.5	3.5

[a] Level of significance ($P \leq 0.05$)

[b] C_1: NMITLI-118, C_2: NMITLI-101, P_1: 2, 50,000 plants ha^{-1}, P_2: 3, 33,333 plants ha^{-1}, P_3: 5, 00,000 plants ha^{-1}, P_4: 10, 00,000 plants ha^{-1}, H_1: Pre- flowering harvest stage, H_2: full flowering harvest stage, H_3: seed maturity harvest stage. DAS: Days after sowing

wider spacing (P_1: 20 × 20 cm) produced a higher number of branches (21.44/plant), leaves (273.45/plant), canopy spread (36.76 cm), and root girth (16.9 mm), owing to reduced inter-plant competition and better availability of resources such as light, water, and nutrients. In contrast, closer spacing (P_4) resulted in taller plants (125.49 cm) with longer roots (16.68 cm), possibly due to increased competition leading to elongation growth.

Overall, cultivar C_1 performed better under summer conditions, with treatment combinations $C_1P_1S_2$ and $C_1P_2S_2$ showing superior growth performance. Growth attributes increased with crop age and were highest at the seed maturity stage (180 DAS), indicating continued biomass accumulation over time. These findings are in agreement with previous reports in *Andrographis paniculata*[20].

A continuous increase in the number of leaves (252.84 and 276.76/plant) and leaf area index (LAI: 4.71 and 5.76) was observed at the pre-flowering and full flowering stages, respectively. However, a decline in the number of leaves (260.46/plant) and LAI (5.58) was recorded at the seed maturity stage.

The growth parameters of *Withania somnifera* (L.) Dunal were significantly influenced by the interaction of seasonal variation, cultivars, plant spacing, and harvest stages. Among the treatments, the highest values were recorded in cultivar NMITLI-118 under plant spacing P_1, particularly at the pre-flowering (90 DAS; H1) and full flowering (H2) stages of harvest.

Evaluation of photosynthetic pigments

Based on the mean data of both years, S_2 (summer season) was found to be superior to S_1 (winter season) with respect to photosynthetic parameters, including chlorophyll content, total chlorophyll, chlorophyll fluorescence, and carotenoids. The data presented in Fig. 3 and 4 indicate that the highest contents of chlorophyll *a* (1.87 mg/g fresh leaf weight), chlorophyll *b* (0.58 mg/g fresh leaf weight), total chlorophyll (2.24 mg/g fresh leaf weight), chlorophyll fluorescence parameters, i.e., Fv/Fm (0.92) and Fv/Fo (7.04), and carotenoids (0.47 mg/g fresh leaf weight) were recorded in cultivar C_1 (cv. NMITLI-118).

Environmental factors such as temperature, humidity, and duration and intensity of sunlight significantly influenced photosynthetic pigments. The

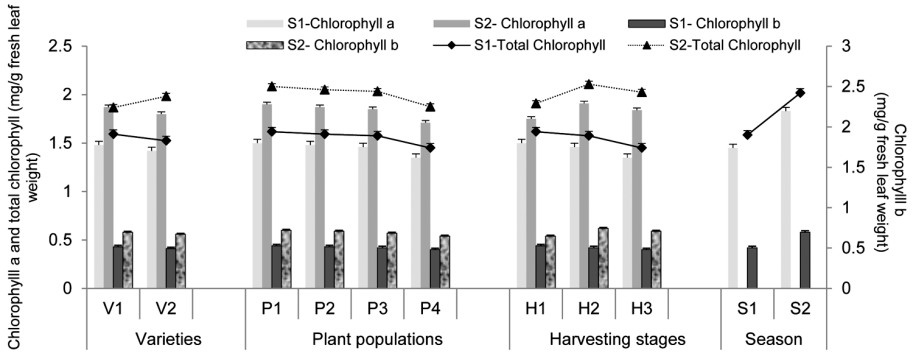

Fig. 3 — Effects of different cultivars, plant populations, and stages of harvest on chlorophyll a, b and total chlorophyll (mg/g fresh leaf weight), in *Withania somnifera* (mean of two-year data).

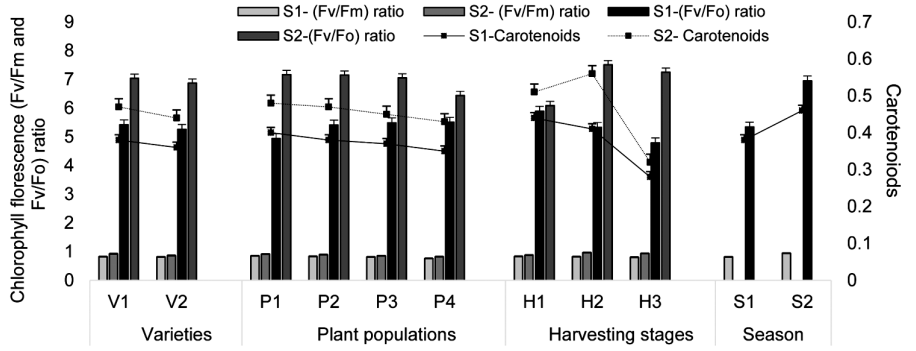

Fig. 4 — Effects of different cultivars, plant populations and stages of harvest on chlorophyll fluorescence (Fv/Fm) ratio, (Fv/Fo) ratio, and carotenoids in *Withania somnifera* (mean of two-year data).
[a] Level of significance ($P \leq 0.05$). C_1: NMITLI-118, C_2: NMITLI-101, P_1: 2, 50,000 plants ha^{-1}, P_2: 3, 33,333 plants ha^{-1}, P_3: 5, 00,000 plants ha^{-1}, P_4: 10, 00,000 plants ha^{-1}, H_1: Pre- flowering harvest stage, H_2: full flowering harvest stage, H_3: seed maturity harvest stage, S_1: Winter season, S_2: Summer season.

summer season combined with P_1 crop spacing (20 cm × 20 cm) resulted in the maximum values of chlorophyll *a* (1.90 mg/g fresh leaf weight), chlorophyll *b* (0.60 mg/g fresh leaf weight), total chlorophyll (2.50 mg/g fresh leaf weight), chlorophyll fluorescence (Fv/Fm = 0.91 and Fv/Fo = 7.17), and carotenoids (0.48 mg/g fresh leaf weight), compared to the winter season and wider spacings (P_2, P_3, and P_4).

Optimum plant spacing plays a crucial role in the efficient utilisation of natural resources. An ideal plant population ensures adequate spacing to maximise light interception and efficient utilisation of photosynthetically active radiation (PAR) by individual plants. Wider spacing enhances chlorophyll content by improving light penetration into the lower canopy. In contrast, the highest plant density, P_4 (1,000,000 plants/ha), recorded the lowest levels of chlorophyll, chlorophyll fluorescence, and carotenoids. This reduction may be attributed to a decrease in chloroplast number and grana lamellae in mesophyll cells with increasing plant density and crop age, which directly affects pigment accumulation[21].

In this series, concerning harvest stages, a continuous pattern of increasing photosynthetic pigments from the preflowering stage (90 DAS) to full flowering (120 DAS), followed by a decreasing trend at seed maturity (180 DAS) was observed. The maximum chlorophyll a (1.75 and 1.91 mg/g fresh leaf weight), chlorophyll b (0.54 and 0.62 mg/g fresh leaf weight), total chlorophyll (2.29 and 2.53 mg/g fresh leaf weight), chlorophyll fluorescence i.e., Fv/Fm (0.87 and 0.93) ratio, Fv/Fo (6.09 and 7.51) ratio, and carotenoids (0.51 and 0.56) content recorded at pre-flowering (90 DAS) as well as full flowering stage of harvest (120 DAS) in summer season than winter season. Generally, pigment synthesis peaked at a specific stage of plant growth and then declined as the plant aged. This could be due to a decline in the plant's nutrient supply or to a leaf approaching senescence. The minimum values of the above-mentioned photosynthetic pigments were reported in cultivar C2 at plant population density

(P4), especially when harvested at seed maturity (180 DAS) in both summer and winter seasons. The interaction effect of cultivar, plant population, and harvest stage indicated that maximum chlorophyll content (chlorophyll a, b, and total), chlorophyll fluorescence (Fv/Fm and Fv/Fo), and carotenoids were recorded in cultivar C_1 under closer spacing (P_1: 33,333 plants/ha) at the seed maturity stage during the summer season. This may be attributed to higher canopy density and enhanced light interception, leading to improved photosynthetic efficiency. However, relatively wider spacing (P_2) often resulted in higher yields due to reduced interplant competition, improved resource availability, and enhanced root development, highlighting a balance between physiological efficiency and yield optimisation.

Evaluation of biological yields

Based on the pooled mean data of both years, S_2 (summer season) was found to be superior to S_1 (winter season) with respect to biological yields, including dry leaves, dry roots, and seeds. The data presented in Tables 5 and 6 indicate that yields of dry leaves, roots, and seeds in two ashwagandha cultivars were significantly influenced by seasonal variation, plant population, and harvesting stage.

The highest yields of dry leaves (1.94 t/ha), dry roots (1.62 t/ha), and seeds (1.01 t/ha) were recorded in cultivar C_1 grown during the summer season. These findings are in agreement with earlier reports[22-29].

In contrast, cultivar NMITLI-101 (C_2) recorded comparatively lower yields of dry leaves (1.66 t ha^{-1}), dry roots (1.32 t/ha), and seeds (0.83 t/ha across both growing seasons.

Irrespective of harvesting stages, the maximum dry leaves yield (2.00 t/ha) and dry root yield (1.94 t/ha) were obtained at the full flowering stage (120 DAS); however, no seed yield was recorded at this stage.

Plant spacing also significantly influenced biological yields. The highest dry leaves yield (2.04 t/ha), dry root yield (1.74 t/ha), and dry seed yield (0.67 t/ha were achieved at a spacing of 20 cm × 15 cm (P_2).

The significant impacts of plant population density, cultivars, harvesting stage, etc., on various yield

Table 5 — Effects of different cultivars; plant populations and stages of harvest on fresh leaves yield (t/ha), dry leaves yield (t/ha), fresh root yield (t/ha), and dry root yield (t/ha) in *Withania somnifera* (mean values of the two years data)

Cropping period	Fresh leaves yield (t/ha)		Dry leaves yield (t/ha)		Fresh root yield (t/ha)		Dry root yield (t/ha)	
Treatments	Winter season (S_1)	Summer season (S_2)	Winter season (S_1)	Summer season (S_2)	Winter season (S_1)	Summer season (S_2)	Winter season (S_1)	Summer season (S_2)
				Cultivars				
(C_1)	3.94	6.57	1. 20	1.94	3.32	5.56	1.04	1.62
(C_2)	3.49	6.02	1.00	1.66	2.80	4.49	0.86	1.32
CD at 5%	0.11	0.48	0.05	0.10	1.21	0.34	0.04	0.08
				Plant spacing (cm)				
(20×20cm) (P_1)	3.74	6.24	1.13	1.89	3.14	5.19	1.00	1.54
(20×15cm) (P_2)	4.13	6.89	1.29	2.04	3.45	5.81	1.11	1.74
(20×10cm) (P_3)	3.61	6.04	1.01	1.72	2.93	4.66	0.85	1.32
(20×05cm) (P_4)	3.38	6.01	0.98	1.55	2.83	4.44	0.84	1.27
CD at 5%	0.15	0.67	0.07	0.14	1.71	0.47	0.06	0.11
				Harvesting stages (DAS)				
(90DAS) (H_1)	3.40	6.26	0.91	1.60	2.47	3.68	0.73	1.08
(120DAS) (H_2)	4.11	6.86	1.31	2.00	3.70	6.54	1.20	1.94
(180 DAS) (H_3)	3.71	5.77	1.09	1.79	3.01	4.86	0.92	1.38
CD at 5%	0.13	0.58	0.06	0.12	0.15	0.41	0.05	0.10
				Interactions				
	CD at 5%	CD at 5%	CD at 5%	CD at 5%	CD at 5%	CD at 5%	CD at 5%	CD at 5%
C×P	0.21	0.95	0.10	0.19	0.24	0.67	0.09	0.16
C×H	0.18	0.82	0.09	0.17	0.21	0.58	0.07	0.14
P×H	0.26	1.16	0.13	0.24	0.30	0.82	0.10	0.20
C×P×H	0.37	1.65	0.18	0.33	0.42	1.16	0.15	0.28

[a] Level of significance ($P \leq 0.05$)

[b] C_1: NMITLI-118, C_2: NMITLI-101, P_1: 2, 50,000 plants ha^{-1}, P_2: 3, 33,333 plants ha^{-1}, P_3: 5, 00,000 plants ha^{-1}, P_4: 10, 00,000 plants ha^{-1}, H_1: Pre- flowering harvest stage, H_2: full flowering harvest stage, H_3: seed maturity harvest stage. DAS: Days after sowing

Table 6 — Effects of different cultivars, plant populations, and only seed maturity stage of harvest on fresh seed yield (t/ha), and dry seed yield (t/ha) in *Withania somnifera* (mean values of the two years data)

Cropping Period	Fresh seed yield (t/ha)		Dry seed yield (t/ha)	
Treatments	Winter season (S_1)	Summer season (S_2)	Winter season (S_1)	Summer season (S_2)
		Cultivars		
NMITLI-118 (C_1)	0.71	1.01	0.33	0.71
NMITLI-101 (C_2)	0.58	0.83	0.10	0.58
SEm ±	0.02	0.03	0.01	0.02
CD at 5%	0.07	0.09	0.03	0.07
		Plant spacing (cm)		
(20×20cm) (P_1)	0.61	0.91	0. 31	0.66
(20×15cm) (P_2)	0.67	0.96	0.32	0.67
(20×10cm) (P_3)	0.63	0.94	0.30	0.64
(20×05cm) (P_4)	0.61	0.86	0.28	0.61
SEm ±	0.03	0.04	0.01	0.03
CD at 5%	NS	NS	NS	NS
		Interactions		
	SEm ± / CD at 5%	SEm ± / CD at 5%	SEm ± / CD at 5%	SEm ± / CD at 5%
C×P	0.60 / *NS*	0.19 / *NS*	0.19 / *NS*	0.27 / *NS*

[a] Level of significance ($P \leq 0.05$), *NS*=non-significant.
[b] C_1: NMITLI-118, C_2: NMITLI-101, P_1: 2, 50,000 plants ha^{-1}, P_2: 3, 33,333 plants ha^{-1}, P_3: 5, 00,000 plants ha^{-1}, P_4: 10, 00,000 plants ha^{-1}, H_1: Pre- flowering harvest stage, H_2: full flowering harvest stage, H_3: seed maturity harvest stage. DAS: Days after sowing

parameters have also been reported in *Ocimum basilicum*[30]. There was a significant difference in yields among various harvesting stages. However, dry leaf and root yields increase from pre-flowering (90 DAS) to the full flowering stage (120 DAS). In contrast to the higher seed yield (0.22 t/ha) at the successive later stage (seed maturity-180 DAS), the lower dry leaves (1.60 t/ha) and root yield (1.08 t/ha) were obtained at pre-flowering stages of harvesting (90 DAS). The interaction effect of cultivars, plant populations, and stages of harvesting also indicated that the maximum dry leaves, dry root, and dry seed yield was recorded with cultivars (C_1), while (P_2) plant population and harvesting were done at the full flowering stage (120 DAS) (H_2). While summer-season-grown crops produced higher seed yields than winter-season-grown crops harvested at maturity (180 DAS). The $C_1 \times P_2 \times H_2$ treatment combination shows better interaction effects than all other treatment combinations.

Evaluation of quality parameters

Data on withanolide content, starch content (%), crude fibre (%), and starch: fibre ratios observed in the root part of the plants are shown in Figs. 5 and 6. Seasonal variations, plant population density, and stages of harvesting showed a significant impact on withanolide content, withanolide yield, starch content (%), crude fibre (%), and starch: fibre ratio of two cultivars (NMITLI-101 & NMITLI-108) of ashwagandha. Data obtained from the mean data of the season from the years, withanolide content (0.35 %) and total withanolide yield (4.92 kg/ha), starch content (25.32 %), crude fibre (37.54%), and starch: fibre (0.80) ratio were recorded as higher in cultivar (C_1). Seasonal variation significantly influences physiological processes, leading to the accumulation of secondary metabolites. The summer season recorded higher secondary metabolite content and yield, likely due to enhanced metabolic activity during this period. Similar findings have been reported in *Withania somnifera*, supporting the present observations[26] regarding plant population; the 20 cm × 15 cm spacing (P_2) resulted in higher withanolide content (0.35%) and total withanolide yield (5.23 kg/ha). This treatment also recorded higher starch content (24.27%), crude fibre content (39.57%), and a starch: fibre ratio (0.94) than other population densities.

Wider spacing yielded higher, in *Chamomilla recutita* (L) Rausch[31] and in *Ocimum sanctum*[15]. While closer spacing restricts the amount of water and nutrients available, this stunts plant growth and inadvertently stresses plants. In times of stress, plants produce more secondary metabolites to cope with unfavourable conditions. Therefore, reducing plant populations can enhance root crops by boosting root weight and biomass, as well as their quality.

Similarly, harvesting stages possess a significant effect on several chemical compounds. The withanolide content (0.36 %), withanolide yield

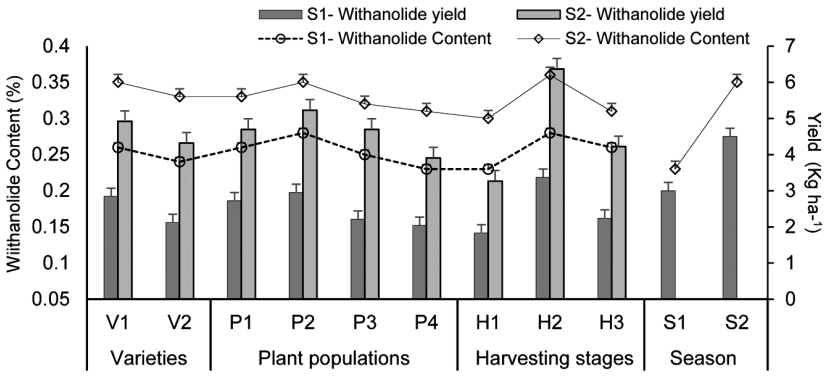

Fig. 5 — Effects of different cultivars, plant populations and stages of harvest on withanolide content (%), and total withanolide yield (kg ha^{-1}) in *Withania somnifera* (mean of two year data). [a]Level of significance ($P \leq 0.05$). C_1: NMITLI-118, C_2: NMITLI-101, P_1: 2, 50,000 plants ha^{-1}, P_2: 3, 33,333 plants ha^{-1}, P_3: 5, 00,000 plants ha^{-1}, P_4: 10, 00,000 plants ha^{-1}, H_1: Pre- flowering harvest stage, H_2: full flowering harvest stage, H_3: seed maturity harvest stage, S_1: Winter season, S_2: Summer season.

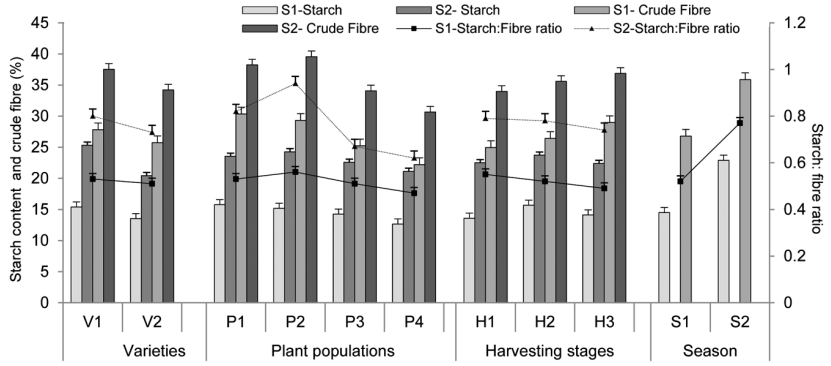

Fig. 6 — Effects of different cultivars; plant populations and stages of harvest on Starch content %, Crude Fibre % and Starch: Fibre Ratio in *Withania somnifera* (mean of two year data). [a]Level of significance ($P \leq 0.05$). C_1: NMITLI-118, C_2: NMITLI-101, P_1: 2, 50,000 plants ha^{-1}, P_2: 3, 33,333 plants ha^{-1}, P_3: 5, 00,000 plants ha^{-1}, P_4: 10, 00,000 plants ha^{-1}, H_1: Pre- flowering harvest stage, H_2: full flowering harvest stage, H_3: seed maturity harvest stage, S_1: Winter season, S_2: Summer season.

(6.37 kg/ha), starch content (23.73 %), and starch: fibre ratio (0.78) were observed to be highest at the full flowering stage of harvesting (120DAS). While crude fibre content was higher (36.68%) at the seed-maturity stage of harvesting. In *Fritillaria cirrhosa*, the concentration of alkaloids was higher during the early stages of fruit development and dramatically reduced as the fruit reached the maturity stage[32]. While the low concentration of withanolide content (0.30%) and withanolide yield (3.27 kg/ha), starch content (22.50%), crude fibre (33.98%), and starch: fibre ratio (0.79) at the pre-flowering harvesting stage (90 DAS), may be due to the pre-maturity at vegetative phase of the plant with the lower synthesizes of secondary metabolites and chemical compounds. *Pennisetum pedicellatum* Trin. and ashwagandha were significantly impacted by harvesting age[33,34]. The researchers discovered that the antioxidant activity varied greatly and affected the quantity of polyphenol as a result of planting and harvesting time variations. Similarly, the effects of several harvesting stages (before flowering, early flowering, full flowering, and fruit. Among the different plant spacings, 20 × 15 cm (P_2) recorded superior performance due to its ability to maintain an optimum plant population that balanced inter-plant competition and efficient resource utilisation. This spacing ensured better light interception, enhanced canopy development, improved root growth, and greater photosynthetic efficiency, resulting in higher herbage yield and essential oil production. In contrast, closer spacing increased competition for light, water, and nutrients, whereas wider spacing led to underutilization of available resources. Furthermore, harvesting at the full flowering stage (120 DAS) resulted in maximum biomass and essential oil accumulation owing to peak physiological and metabolic activity. Seasonal variation also indicated that summer conditions favoured greater vegetative

Table 7 — Cost of cultivation, gross return, net return and benefit-cost (B: C) ratio as influenced by cultivars, plant population, and stages of harvest in *Withania somnifera* (mean values of two years data).

Cropping period	Cost of cultivation (US$ ha^{-1})		Gross return (US$ ha^{-1})		Net return (US$ ha^{-1})		Benefit-cost (B:C) ratio	
Treatments	Winter season (S_1)	Summer season (S_2)	Winter season (S_1)	Summer season (S_2)	Winter season (S_1)	Summer season (S_2)	Winter season (S_1)	Summer season (S_2)
				Cultivars				
(C_1)	469.57	483.66	2462.99	2774.35	1993.42	2290.69	4.25	4.74
(C_2)	453.71	467.32	2033.63	2299.06	1579.92	1831.74	3.48	3.92
				Plant spacing (cm)				
(20×20cm) (P_1)	457.37	471.09	2355.71	2654.41	1898.34	2183.32	4.15	4.63
(20×15cm) (P_2)	463.47	477.37	2623.92	2965.89	2160.45	2488.51	4.66	5.21
(20×10cm) (P_3)	434.48	447.52	2018.44	2292.91	1583.96	1845.40	3.65	4.12
(20×05cm) (P_4)	432.70	445.68	1994.33	2234.57	1561.64	1788.89	3.61	4.01
				Harvesting stages (DAS)				
(90DAS) (H_1)	341.51	351.76	1764.95	1975.05	1423.44	1623.29	4.17	4.61
(120DAS) (H_2)	490.91	505.64	2796.77	3194.55	2305.86	2688.91	4.70	5.32
(180 DAS) (H_3)	536.40	552.50	2183.94	2442.14	1647.54	1889.65	3.07	3.42

[a] the market price has been taken mean value of all experimental years.
[b] 1 US$ = 82.78 INR.
[c] Current market price of Ashwagandha dry roots = Rs.150 or 1.83 US$ kg^{-1}
[d] Current market price of Ashwagandha dry leaves = Rs.40 or 0.48 US$ kg^{-1}

growth, essential oil synthesis, and overall yield compared to winter conditions[35].

Evaluation of economics

Table 7 shows the mean data obtained from both seasons, indicating that the highest cultivation cost (476.62 US$/ha) was observed in *cv.* NMITLI-118 (C_1), with (P_2) (470.42 US$/ha) plant spacing at the seed maturity stage (180 DAS) (544.45 US$ ha^{-1}) of harvesting. In contrast, the lowest cultivation cost (460.52 US$ ha^{-1}) was found in cultivar (C_2), regardless of the plant population P_4 (1994.33 US$/ha) at the pre-flowering (346.64 US$/ha) harvesting stage (90DAS) (H_1). It was due to insufficient plant growth, with low resource consumption. Yield per plant reduced dramatically as plant density increased[36].

The maximum gross returns (2618 US$/ha) and net returns (2142.06 US$/ha) were recorded in the treatment combination with cv. NMITLI-118 (C_1), along with plant spacing (P_2), the gross return (2794.91 US$/ha) and net return (2324.48 US$/ha), at the full flowering stage of harvesting, the gross return (2995.66 US$/ha) and net return (2497.39 US$/ha), respectively.

The benefit-cost ratio is an essential economic parameter for crop yield. Fig. 6 shows the benefit-cost ratio of all treatment combinations, indicating that the maximum benefit-cost ratio (5.21) was computed with plant populations (P_2) at the full flowering (5.32) stage of harvesting with C_1 (4.74) cultivars in the summer season. Maximum yield of leaves, roots, and seeds in *W. somnifera* was obtained at 20 cm × 15 cm spacing (P_2) with harvesting at 120 DAS under cultivar C_1 (NMITLI-118). This spacing ensured an optimal plant population and efficient resource use, balancing plant density and individual growth. Closer spacing increased competition, while wider spacing reduced overall yield. Harvesting at full flowering enhanced root biomass and withanolide accumulation by improving assimilate partitioning. Thus, optimum spacing combined with appropriate harvest timing significantly improved yield and quality. As a result of assessing the entire findings, several ashwagandha cultivars showed beneficial interactions with the summer season, plant population, and harvesting stages, and proved to be more lucrative than the control. However, growing ashwagandha as an off-season crop provided natural conditions that increased yield production and generated monetary gain, particularly for small land-holding farmers, and allowed for crop diversification with the least chance of crop failure.

Conclusion

The study revealed that cultivar C_1 (NMITLI-118), grown during the summer season at a spacing of 20 × 15 cm and harvested at 120 DAS, produced the highest yield and superior quality in *W. somnifera*. Summer cultivation was found to be suitable due to

favourable biochemical changes and enhanced accumulation of secondary metabolites. The crop requires relatively fewer inputs and has a short duration (90–100 days), making it an efficient option for off-season cultivation under irrigated conditions in the northern Indian plains. Cultivation of Ashwagandha as a summer crop can promote crop diversification, reduce the risk of crop failure, and increase land-use efficiency, particularly in fields left fallow between the Kharif and Rabi seasons due to biotic and abiotic constraints, such as grazing and untimely rainfall. This practice can enhance farmers' incomes during the lean period, generate additional employment, and increase area and production, ensuring the availability of high-quality raw materials for the pharmaceutical industry at lower cost.

Conflict of interest

The authors declare that they have no known monetary or other personal interests, direct or indirect, in any matter that raises a conflict with this paper.

AI use disclosure

The authors used an artificial intelligence (AI)-assisted language tool to improve the readability, grammar, and clarity of the manuscript and to rephrase certain sections of the text. The AI tool was used solely for language editing purposes and did not contribute to the study design, data collection, data analysis, interpretation of results, or scientific conclusions. All content was carefully reviewed, verified, and approved by the authors, who take full responsibility for the accuracy and integrity of the manuscript.

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