



# **EFFECT OF INTEGRATED NUTRIENT MANAGEMENT SYSTEM ON TOTAL ALKALOID CONTENT IN DIFFERENT PLANT PARTS OF ASHWAGANDHA (*WITHANIA SOMNIFERA* (L.) DUNAL)**

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## **Abstract**

Ashwagandha (*Withania somnifera* (L.) Dunal) is an important medicinal plant of the family solanaceae which contains several alkaloids in different plant parts. Therefore, the present study was conducted to know the effect of integrated nutrient management system on total alkaloid content in root, stem, leaf and fruit of Ashwagandha at near the college of agriculture farm, Tikamgarh (M.P.) India during kharif season of 2017-18 and 2018-19. The experiment was laid out in randomized block design with three replications and twelve treatment viz, 100% NPK recommended dose 50:30:30 kg/ha (T<sub>1</sub>), 100% NPK/ha + 5 kg Zn/ha (T<sub>2</sub>), 10 tonnes FYM/ha (T<sub>3</sub>), 10 tonnes FYM/ha + 3 kg PSB/ha (T<sub>4</sub>), 10 tonnes FYM/ha + 3 kg Azotobacter/ha (T<sub>5</sub>), 10 tonnes FYM/ha + 3 kg Azotobacter/ha + 3 kg PSB/ha (T<sub>6</sub>), 10 tonnes FYM/ha + 3 kg Azotobacter/ha + 3 kg PSB/ha + 5 kg Zn/ha (T<sub>7</sub>), 50% NPK/ha + 5 tonnes FYM/ha (T<sub>8</sub>), 50% NPK/ha + 5 tonnes FYM/ha + 3 kg PSB/ha (T<sub>9</sub>), 50% NPK/ha + 5 tonnes FYM/ha + 3 kg Azotobacter/ha (T<sub>10</sub>), 50% NPK/ha + 5 tonnes FYM/ha + 3 kg Azotobacter/ha + 3 kg PSB/ha (T<sub>11</sub>) and 50% NPK/ha + 5 tonnes FYM/ha + 3 kg Azotobacter/ha + 3 kg PSB/ha + 5 kg Zn/ha (T<sub>12</sub>). The pooled analysis of two years data indicated that integrated nutrient management system significantly influenced total alkaloid content in different parts of Ashwagandha. The significant highest values of total alkaloid content were recorded in root (0.564%), stem (0.192%), leaf (0.341%) and fruit (0.131%) with the application of 50% NPK/ha+5 tonnes FYM/ha+3 kg Azotobacter/ha+3 kg PSB/ha + 5 kg Zn/ha (T<sub>12</sub>). However, lowest values of total alkaloid content in root (0.499%), stem (0.133%), leaf (0.208%) and fruit (0.109%) were recorded with the application of 100% NPK recommended dose 50:30:30 kg/ha (T<sub>1</sub>). Based on overall experimental results, it could be concluded that 50% NPK/ha+5 tonnes FYM/ha+3 kg Azotobacter/ha + 3kg PSB/ha + 5 kg Zn/ha (T<sub>12</sub>) treatment was found to be a better integrated nutrient management system which can be used for better synthesis and accumulation of total alkaloid content in different plant parts of Ashwagandha (*Withania somnifera* (L.) Dunal) crop.

**Keywords:** Integrated nutrient management system, total alkaloid content, Ashwagandha (*Withania somnifera* (L.) Dunal).

## Introduction :

Ashwagandha (*Withania somnifera* (L.) Dunal) is a popular Indian medicinal plant belonging to family solanaceae and is also known as Indian ginseng, poison gooseberry and winter cherry. It is one of the most valuable plant in the traditional. Indian system of medicine (**Alam et. al., 2012**). It is mainly found in Rajasthan, Punjab, Haryana, Uttar Pradesh, Gujarat, Maharastra and Madhya Pradesh (**Chaurasia and Singh, 2022**). In Madhya Pradesh, it is cultivated in Neemuch, Manasa and Jawad tehisils of Mandsaur district (**Sharma, 2013**). It is an erect, evergreen, tomentose shrub with 30 to 120 cm height (**Fig.1**).

**Fig.1** Ashwagandha Plant (*Withania somnifera* (L.) Dunal)



Roots of this plant are stout, fleshy and whitish brown; The leaves are simple, ovate, glabrous: the flowers are inconspicuous, greenish or lurid yellow; the berries are glabose, orange red when mature enclosed in the persistent calyx and have yellow reniform seeds.

The roots of Ashwagandha is pre dominantly used as an aphrodisiac, adaptogenic, immunomodular, anti-inflammatory and geriatric tonic. It is also used for the treatment of rheumatism, cough, arthritis, tiredness, insomnia, muscular strength, stress, memory loss, aging complications, asthma, anxiety, constipation, ulcer, fever, diabetes, nervous breakdowns, sterility and impotence (**Kulkarni and Dhir, 2008; Pandian et al., 2020; Chaurasia and Singh (2022)**).

The root of Ashwagandha and occasionally its leaves, seeds and berries are used in ayurvedic and unani system of medicine. (**Ahmad and Dar, 2017; Behl et al., 2020**).

These medicinal values are attributed to the presence of alkaloids. The important alkaloid present in the roots of Ashwagandha is withanine constituting 38 per cent of the total alkaloids. Other alkaloids recorded are somniferine, somniferinine, somninine, withanine, pseudowithanine, withananinine and withasomine (**Majumdar, 1952; Schwarting et al, 1963; Saleem et al., 2022 Abdelwahed et al., 2023**).

The total alkaloid content of the roots have been reported to vary between 0.13 to 0.31 per cent (**Anon et al, 1976**). A part from roots alkaloids have also been reported in leaves and berries (**Gupta et al., 1996; Sreerekha et al., 2004; Essam et al., 2016; Malviya et al., 2017; Teja et al., 2022**).

Because of its numerous medicinal properties, the Ashwagandha plant is in great demand from both ayurvedic and pharmaceutical industries and needs commercial cultivation to meet its demand. Integrated nutrient management system is the only alternative that may help in enhancing sustained productivity and quality of Ashwagandha.

Integrated nutrient management system is the maintenance and adjustment of soil fertility and of plant nutrient supply to an optimum level for sustaining the desired crop productivity through optimization of the benefits from all possible sources of plant nutrients in an integrated manner (**Chaurasia and Singh, 2021b**). Biofertilizers improve soil fertility and promote plant growth, and they are broadly classified into nitrogen fixers, phosphate solubilizers and phosphate mobilizers and organic matter decomposers. They enhance certain biological processes by which nutritionally important elements are made available to the plants. (**Lian et al., 2002**). Ashwagandha is a non-leguminous crop that hosts colonizing symbiotic nitrogen fixers and phosphate solubilizing bacteria (PSB) in its rhizosphere (**Katznelson, 1965**). The increased uptake of nutrients from soil due to the application of chemical nutrients farm yard manure and biofertilizers might have produced enough carbohydrates in leaves for translocation to the sink for maximum productivity.

Keeping the above facts in view, a present investigation was carried out to study the "Effect of integrated nutrient management system on total alkaloid content in different plant parts of Ashwagandha (*Withania somnifera* (L.) Dunal)".

## **Materials and Methods :**

The details of experimental procedure adopted, materials used and techniques followed during the course of present investigation are described as under.



**Experimental site and location :**

A field experiment was conducted during the Kharif season of 2017-18 and 2018-19 at near the college of agriculture farm, Tikamgarh, Madhaya Pradesh, India which is situated in the Bundelkhand region No. VIII of agroclimatic zone of the state. It lies between the latitude  $24^{\circ} 26' N$  to  $25^{\circ} 40' N$  and longitude  $78^{\circ} 26' E$  to  $79^{\circ} 28' E$  and at the altitude of 426.7 m above mean sea-level (Fig. 2).

Fig.2 A general view of field experiment

**Soil of the experimental field :**

The soil of the experimental field was sandy loam with good drainage capacity. Soil analysis revealed that it has low available nitrogen (189 -191 kg/ha) availability with low available phosphorus (17.9 - 18.1 kg/ha), high in available potassium (298 - 302 kg/ha) and low in available sulphur (15.5 -16.5 kg/ha). The soil was low in DTPA extractable iron (6.6 -6.8 mg/kg), zinc (0.37 -0.41 mg/kg), copper (0.29 -0.31 mg/kg) and manganese (2.7 - 3.1 mg/kg).. The presence of organic carbon was also low (0.43-0.45%) with neutral in reaction (pH 7.4 -7.6). The electrical conductivity of soil was safe (0.33 - 0.37 dS/m). The soil could be related as poor with respect to available nitrogen and organic matter, high with respect to available potash. The soil pH was neutral in reaction.

## Experimental materials :

Jawahar Ashwagandha-20 (JA-20) was used in the present investigation, and it was sown in each experimental plot. Nitrogen (N), Phosphorus (P), Potassium (K), and biofertilizers were applied as Urea, Single superphosphate, muriate of potash and Azotobacter, PSB, respectively. FYM was applied in experimental plots just before sowing.

## Seed sowing :

The healthy and good quality seeds of Ashwagandha (Jawahar Ashwagandha-20) were sown at the rate of 10 kg/ha by hand at a depth of 5 cm in open furrows. Light irrigation was provided immediately after sowing.

## Treatment details :

The total experimental area was 46.0 m × 23.0 m and 5.0 m × 4.0 m net plot size. The total twelve integrated nutrient management treatments were applied randomly and replicate thrice in a randomized block design (Chaurasia and Singh, 2021 a and Chaurasia and Singh, 2021 b; Chaurasia and Singh, 2022). The details of treatment as mentioned in Table 1.

**Table 1 Description of Integrated nutrient management treatments.**

Treatments	Details of treatment applications
T <sub>1</sub>	100% NPK recommended dose 50:30:30 kg/ha
T <sub>2</sub>	100% NPK/ha + 5 kg Zn/ha
T <sub>3</sub>	10 tonnes FYM/ha
T <sub>4</sub>	10 tonnes FYM/ha + 3 kg PSB/ha
T <sub>5</sub>	10 tonnes FYM/ha + 3 kg Azotobacter/ha
T <sub>6</sub>	10 tonnes FYM/ha + 3 kg Azotobacter/ha + 3 kg PSB/ha
T <sub>7</sub>	10 tonnes FYM/ha + 3 kg Azotobacter/ha + 3 kg PSB/ha + 5 kg Zn/ha
T <sub>8</sub>	50% NPK/ha + 5 tonnes FYM/ha
T <sub>9</sub>	50% NPK/ha + 5 tonnes FYM/ha + 3 kg PSB/ha
T <sub>10</sub>	50% NPK/ha + 5 tonnes FYM/ha + 3 kg Azotobacter/ha
T <sub>11</sub>	50% NPK/ha + 5 tonnes FYM/ha + 3 kg Azotobacter/ha + 3 kg PSB/ha
T <sub>12</sub>	50% NPK/ha + 5 tonnes FYM/ha + 3 kg Azotobacter/ha + 3 kg PSB/ha + 5 kg Zn/ha

Abbreviation : N = Nitrogen, P= Phosphorus, K= Potassium Zn= Zinc, FYM= Farm yard manure.

PSB = Phosphate solubilizing bacteria, Kg = Kilogram and ha = Hectare.

## Estimation of total alkaloid content in root, stem, leaf and fruit of Ashwagandha :

### Glassware and distilled water :

Corning glassware and double distilled water were used throughout the present experimentation. All glassware was made free from acids, bases and reducing or oxidizing substances. Residues of acids on glassware were washed off with a concentrated solution of sodium phosphate. (**Association of official agriculture chemists, 1960**), followed by repeated washed off with distilled water.

### Collection of plant samples :

The treatment and replication wise mature root, stem, leaf and fruit samples of Ashwagandha were collected from the experimental field and brought to the laboratory in separate polythene bags. These samples were properly washed with tap water to remove adhering foreign particles, mud, dust etc and air dried in shade at room temperature. Each dried sample was cut into small pieces of 2-3 inches and grinded in an electric grinder. The uniform powder so obtained was sieved twice to remove the coarse particles and stored in plastic containers. Each powdered dried root, stem, leaf and fruit sample was used for extraction and estimation of total alkaloid content.

### Extraction and estimation of total alkaloid content :

The total alkaloid content in root, stem leaf and fruit of Ashwagandha (*Withania somnifera* (L.) Dunal) was estimated by the method of **Mishra (1996)**.

One gram of each powdered plant sample of Ashwagandha was weighted separately and put in stoppered test tube, then added 10 ml chloroform and three drops of ammonia, mixed well and kept overnight. Next day after shaking, the contents were filtered through cotton wool in a small beaker and the residue was washed with chloroform thrice (10 ml each), which ensured complete removal of alkaloids. The extract was dried on water bath and added 10 ml ethyl alcohol and mixed the contents with clean glass rod. Then the liquid portion was evaporated which confirms removal of ammonia and added 10 ml of standard acid solution (0.01 N H<sub>2</sub>SO<sub>4</sub>) in the beaker and warmed slightly to dissolve the alkaloids in acid solution. It was cooled and the unused acid was titrated with standard NaOH (0.01 N) and note down the volume of alkali used in titration. A blank was run to find out the exact volume of acid neutralized by the alkaloids. The total alkaloid content expressed in percentage was calculated by using the following formula :

**Total alkaloid content (%) = 0.415 x Volume of acid consumed by alkaloids.**

### Statistical analysis :

The pooled data were statistically analyzed through Anova technique as suggested by **Fisher and Yates (1963) and Fisher (1967)**.



## Results and Discussion :

The pooled analysis of two years data representing the effect of integrated nutrient management system on total alkaloid content in root, stem, leaf and fruit of Ashwagandha (*Withania somnifera* (L.)Dunal) are given in **Table 2** and **Fig. 3**. Total alkaloid content in different plant parts of Ashwagandha was significantly influenced by different integrated nutrient management system. Highest total alkaloid content in root (0.564%), stem (0.192%), leaf (0.341%) and fruit (0.131%) of Ashwagandha was recorded with 50% NPK /ha +5 tonnes FYM/ha + 3 kg Azotobacter/ha + 3 kg PSB/ha + 5 kg Zn/ha (T<sub>12</sub>) followed by 50% NPK/ha + 5 tonnes FYM/ha + 3 kg Azotobacter/ha + 3 kg PSB/ha (T<sub>11</sub>). Significantly lowest total alkaloid content in root (0.499%), stem (0.133%), leaf (0.208%) and fruit (0.109%) of Ashwagandha was recorded under treatment of 100% NPK recommended dose 50:30:30 kg/ha (T<sub>1</sub>).

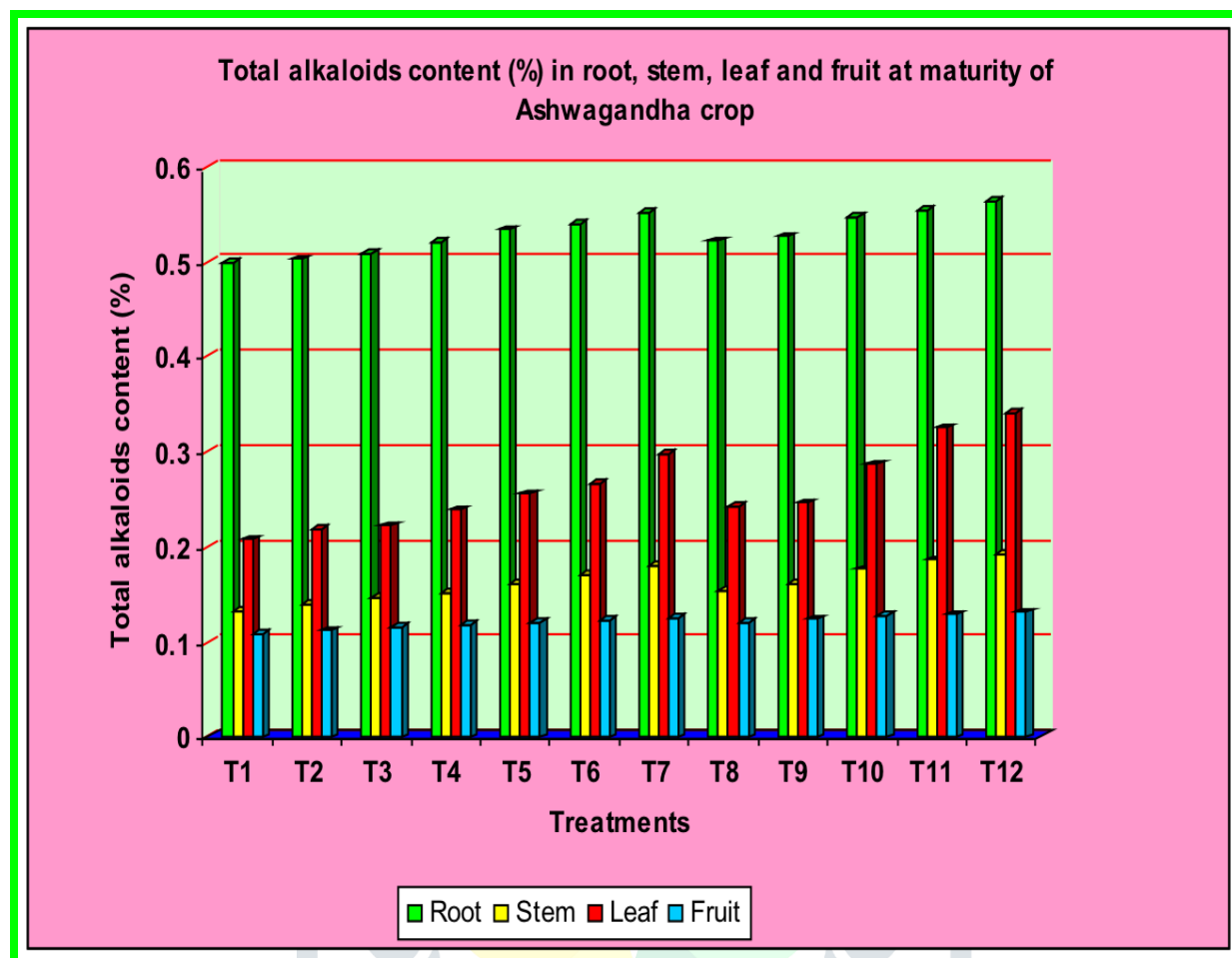
The increase of total alkaloid percentage in root, stem, leaf and fruit of Ashwagandha (*Withania somnifera* (L.) Dunal). is may be due to role of inorganic fertilizers, organic manure and biofertilizers in supporting the plant growth and building a root system which is featured with high quality in absorption the nutritional contents this is reflected on cell divisions and growth and increase the leaf area as well as increase the nutritional contents that are made in leaves such as carbohydrates and proteins that are important to building plant tissues which are reflected increasing the dry weight and plant growth and composing the alkaloid components in different plant parts of Ashwagandha. (Kakaraparth et al., 2013)

**Table : 2 Effect of integrated nutrient management system on total alkaloid content (%) in root, stem, leaf and fruit at maturity of Ashwagandha crop (Mean data of two years)**

Treatments	Total alkaloid content (%)			
	Root	Stem	Leaf	Fruit
T <sub>1</sub>	0.499	0.133	0.208	0.109
T <sub>2</sub>	0.502	0.140	0.218	0.112
T <sub>3</sub>	0.509	0.147	0.222	0.115
T <sub>4</sub>	0.520	0.151	0.239	0.118
T <sub>5</sub>	0.533	0.161	0.256	0.120
T <sub>6</sub>	0.540	0.171	0.267	0.123
T <sub>7</sub>	0.551	0.181	0.297	0.125
T <sub>8</sub>	0.521	0.154	0.243	0.121
T <sub>9</sub>	0.526	0.161	0.246	0.124
T <sub>10</sub>	0.546	0.177	0.287	0.127
T <sub>11</sub>	0.554	0.186	0.325	0.129
T <sub>12</sub>	0.564	0.192	0.341	0.131
SEm±	0.002	0.002	0.004	0.001
CD (P=0.05)	0.006	0.005	0.012	0.002



**Fig. 3 : Total alkaloid content (%) in root, stem, leaf and fruit at maturity of Ashwagandha crop as influenced by integrated nutrient management system. (mean data of two years)**



The inorganic fertilizers, organic manure and biofertilizers and it may be because of its effective role in activating the biological operations and especially photosynthesis which increase the amino acid which are part of preparing alkaloids and increase the percentage in different plant parts of Ashwagandha. The results are in close harmony with the findings of **Maheshwari et al. (2000)**, **Panchabhai et al., (2005)**, **Shrivastava et al., (2012)**, **Goel and Duhan (2014)**, **Patidar et al., (2014)**, **Malviya et al., (2017)** and **Pramod kumar et al. (2019)**. **Maheshwari et al. (2000)** reported that the application of 2.5 tonnes FYM/ha + 12.5 kg N/ha + 25 kg  $P_2O_5$ /ha resulted highest total alkaloid content in root of Ashwagandha. **Panchabhai et al. (2005)** reported that total alkaloid content in root of Ashwagandha was obtained with the treatment NP at 50:50 and 50:75 kg/ha than with other treatments. **Shrivastava et al., (2012)** reported that application of 40:20:20 N:P:K kg/ha + 2.5 tonnes vermicompost/ha + 5 tonnes FYM/ha + 50 kg Zn/ha produced maximum total alkaloid content in Ashwagandha roots. **Goel and Duham (2014)** reported that the significantly highest yield of total alkaloid mg/pot was found in the treatment combination of 12.5 tonnes FYM/ha + inorganic phosphate at the rate of 25 mg  $P_2O_5$ /kg soil as compared with other treatments. **Patidar et al. (2014)** reported that the total alkaloid content in root, stem and leaf of Ashwagandha was recorded significantly maximum with treatment of 50% NPK of recommended dose + 5 tonnes FYM + PSB+ Azotobacter + 5

kg Zn/ha. **Malviya et al. (2017)** reported that the significantly higher values of total alkaloids content in root, stem and leaf of Ashwagandha were observed with treatment 75% NPK of recommended dose + 5 tonnes vermicompost/ha + PSB and Azotobacter 3 kg/ha. **Pramod Kumar et al. (2019)** reported that the maximum total alkaloid content in Ashwagandha was obtained with application of 5 tonnes FYM/ha + 625 g Azotobacter/ha + 625 g Azospirillum/ha + 625g PSB/ha + 25 kg Vam/ha + 3% Panchagavya+ 3% Amruthapani at 15 days intervals upto harvest of crop.

## Conclusion :

Based on the findings of the present study, it can be concluded that the integrated nutrient management system significantly influenced total alkaloid content in different plant parts of Ashwagandha (*Withania somnifera* (L.) Dunal). The highest values of total alkaloid content in root, stem, leaf and fruit of Ashwagandha were recorded with the application of 50% NPK/ha + 5 tonnes FYM/ha + 3 kg Azotobacter/ha + 3 kg PSB/ha + 5 kg Zn/ha (T<sub>12</sub>) when compared to the other treatments. Results of this investigation indicated that 50% NPK/ha + 5 tonnes FYM/ha + 3 kg Azotobacter/ha + 3 kg PSB/ha + 5 kg Zn/ha (T<sub>12</sub>) was found to be a better integrated nutrient management system that can be used for better synthesis and accumulation of total alkaloid content in different plant parts of Ashwagandha (*Withania somnifera* (L.) Dunal) crop.

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