INTERNATIONAL JOURNAL FOR INNOVATIVE RESEARCH IN MULTIDISCIPLINARY FIELD
ISSN(O): 2455-0620 [Impact Factor: 9.47]
Monthly, Peer-Reviewed, Refereed, Indexed Journal with IC Value : 86.87
Volume - 10, Issue - 5, May - 2024

DOIs:10.2015/IJIRMF/202405035 --:-- Research Paper / Article / Review

Effect of Integrated Nutrient Management System on Withanolides Content in Ashwagandha (*Withania somnifera* (L.) Dunal)

Sushmita Chaurasia[1] and Shubha Chaurasia[2]

[1]Department of Botany, Pandit Deendayal Upadhyay, Govt. College, Begamganj 464881, District Raisen (M.P.) India
[2]Department of Botany, Govt. P.G. College, Tikamgarh 472001 (M.P.) India
Email : [1]chaurasiasushmita9@gmail.com, [2]shubhachaurasia415@gmail.com

Abstract: Ashwagandha (*Withania somnifera* (L.) Dunal) is a very unique medicinal plant explored by number of orthodox medicinal systems such as Ayurvada, Siddha and Unani. It is a treasure house of wide spread array of bioactive plant metabolites of which withanolides are the most important ones. Threfore, the present study was conducted to know the effect of integrated nutrient management system on withanolides content in Ashwagandha (*Withania somnifera* (L.) Dunal). A field experiemnt was conducted 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 treatments viz., 100% NPK recommended dose 50:30:30 kg/ha (T_1), 100% NPK/ha + 5 kg Zn/ha (T_2), 10 tonnes FYM/ha (T_3), 10 tonnes FYM/ha + 3 kg PSB/ha (T_4), 10 tonnes FYM/ha + 3 kg Azotobacter/ha (T_5), 10 tonnes FYM/ha + 3 kg Azotobacter/ha + 3 kg PSB/ha (T_6), 10 tonnes FYM/ha + 3 kg Azotobacter/ha + 3 kg PSB/ha + 5 kg Zn/ha (T_7), 50% NPK/ha + 5 tonnes FYM/ha (T_8), 50% NPK/ha + 5 tonnes FYM/ha + 3 kg PSB/ha (T_9), 50% NPK/ha + 5 tonnes FYM/ha + 3 kg Azotobacter/ha (T_{10}), 50% NPK/ha + 5 tonnes FYM/ha + 3 kg Azotobacter/ha + 3 kg PSB/ha (T_{11}) and 50% NPK/ha + 5 tonnes FYM/ha + 3 kg Azotobacter/ha + 3 kg PSB/ha + 5 kg Zn/ha (T_{12}). The pooled analysis of two years data indicated that integrated nutrient management system significantly influenced total withanolides content, withaferin A, withanolide A and withanoliede B content in Ashwagandha. The results revealed that highest values of total withanolides content in root (0.707%), stem (0.202%), leaf (0.595%) and withaferin A content in root (0.051%) were recorded with 50% NPK/ha+5tonnes FYM/ha+3 kg Azotobacter/ha+3 kg PSB/ha+5kg Zn/ha (T_{12}).While highest values of withanolide A (0.039%) and withanolide B (0.037%) content in root were recorded under treatment of 100% NPK recommended dose 50:30:30 kg/ha (T_1). Based on overall expermental results, it could be concluded that 50% NPK/ha+5tonnes FYM/ha+3 kg Azotobacter/ha+ 3kg PSB/ha + 5kg Zn/ha (T_{12}) was found to be better inegrated nutrient management system for better synthesis and accumulation of total withanolides and withaferin A content in Ashwagandha where as, better synthesis and accumulation of withanolide A and withanolied B content was found in the treatment of 100% NPK recommended dose 50:30:30 kg/ha (T_1).

Key Words: Integrated nutrient management system, Ashwagandha (*Withania somnifera* (L.) Dunal), Total withanolides content, withaferin A, withanolide A and withanolide B content.

1. INTRODUCTION :

Ashwagandha (*Withania somnifera* (L.) Dunal) is an evergreen shrub, commonly known as Indian ginseng, poison goosebarry and winter cherry that belongs to the solanaceae family **(Fig.1).** It is used extensively as an herbal drug in the Ayurvedic and unani systems of medicine for the last 300 years **(Ahmad and Dar, 2017; Behl et al. 2020; Chaurasia and Singh, 2022)**.

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

It grows in arid areas of India, Afghanistan, Baluchistan, Pakistan, Sri Lanka, China, Nepal, Congo, South Africa, Egypt, Morocoo and Jordan. It is grown in gardens in warmer parts of Europe and has emerged as a natural weed in South Australia and New South Wales **(Paul et al. 2021)**. In India, it is mostly grown for its fleshy roots, which contain a profusion of phytoconstituents with a multitude of therapeutic values. The plant is widesperead in Indias and areas notably in Punjab, Gujrat, Uttar Pradesh, Madhya Pradesh, Maharashtra, West Bengal and Rajasthan **(Uddin et al. 2012; Chaurasia and Singh, 2022)**. It has been used in traditional system of medicine as an anti-stress, narcotic, diuretic, combating anaemia, aphrodisiac etc. for constipation against worms, liver disease, leprosy, anti inflammatory, cardio vascular problems, joint pain, anti-bacterial, nrevous system disorders, arthritis, asthma, anxiety, ulcer, sterility and impotence (**Behl et al. 2020; pandian et al. 2020; saleem et al. 2020; paul et al. 2021; chaurasia and Singh 2022, Chaurasia and Chaurasia, 2024**).

Various pharmacological activities have been reported in Ashwagandha including anti-inflammatory, analgesic, hemopoestic, anti-stress, anti-tumor, anti-arthritic, hapatoprotective, anti-cancer, anti-epileptic, anti-alzheimer, anti-parkinson, cordioprotective, neuroprotective, anti-bacterial, anti-fungal, anti oxidant, immunomodulatory, anti-depressant, anti-diabetic, anti-platelet, fibrinolytic etc. **(Ku et al., 2014; Kumar et al., 2015; Paul et al., 2021; Parihar, 2022)**.

These pharmacological activities are attributed to the presence of alkaloids and withanolides (steroidal lactones with argostane skeleton). The withanolides have C28 steroidal nucleus with C9 side chain having six membered lactone ring. The basic structure is designated as the withanolide skaleton. **(Fig. 2A)**. A total of 43 withanolides have been

identified in Ashwagandha roots **(Trivedi et al., 2017)**. A part from roots withanolides have also been reported in stems, leaves flowers and fruits **(Chourasia et al., 2008; Patidar et al., 2014; Gajbhiye et al., 2015; Malviya et al., 2017 Singh et al, 2023)** The most importent withanolides present in the Ashwagandha are withaferin A, Withanolide A and withanolide B which are responsible for various bio efficacies. **(Sangwan et al., 2008; Chatterjee et al., 2010; Saleem et al., 2020; Paul et al., 2021).** Chemical structure of these three important withanolides of Ashwagandha is shown in **Fig 2 B, C and D**.

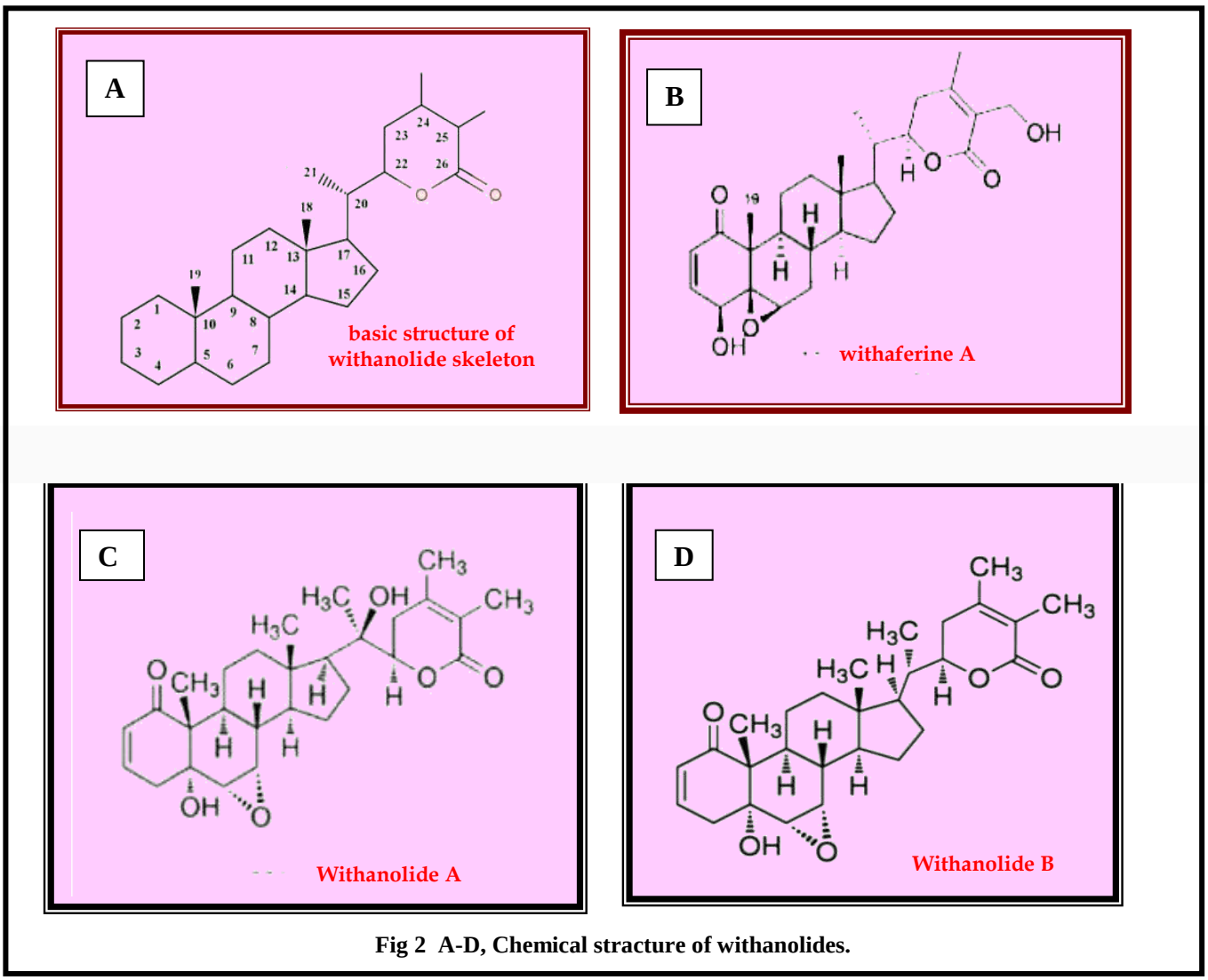


Fig 2 A-D, Chemical stracture of withanolides.

Integrated nutrient management system refers to the maintenance of soil fertility and of plant nutrient supply at an optimum level for sustaining the desired productivity through management of all the sources of organic, inorganic and biological components in an integrated manner **(Chaurasia and Chaurasia, 2024).** The growth, yield, Quality and biochemical content of Ashwagandha largly depend on number of interacting factors. Among them integrated nutrient management system is the most crucial as well as basic factor. The continuous use of chemical fertilizer increases the concentration of heavy metals in the soil, disturbs soil health and quality which can not support plant growth in long term basis. Integrated nutrient management system comprises organic, inorganic component and micro-organism that are highly beneficial for sustainable crop production as it ameliorates soil environment, maintains adequate level of nutrients and provides favourable conditions for high Ashwagandha yield, quality and desired biochemical constituents. Organic manure particularty farmyard manure (FYM) not only balance the nutrient supply but also improve the physical, chemical and biological properties of soil **(Mengistu and Mekonnen, 2012; Chaurasia and Singh, 2022).** Biofertilizers, which are eco-friendly and more economical can play an important role in reducing the dependence on chemical fertilizers. Azotobacter is a free living nitrogen fixing bacteria it has been reported to fix about 20 kg. Nitrogen (N) per hactare per year in a field of non legume crop and also secrets some growth promoting substances, which could stimulate plant growth, absorption of nutrients and photosynthesis. **(Suba Rao, 1982, Mady and Youssef, 2014).**

Phosphate solubilizing bacteria (PSB) particularly the soil bacteria belonging to the genera *Pseudomonas* and *Bacillus* possess the capability to transform insoluble phosphates into soluble forms **(Alexander, 1977).**

Considering the importance of Ashwagandha and role of integrated nutrient management system, the present investigation was carried out to study the effect ot integrated nutrient management system on withanolides content in Ashwagandha (*Withania somnifera* (L.) Dunal).

2. MATERIALS AND METHODS :

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

2.1 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 24^0 26' N to 25^0 40' N and longitude 78^0 26' E to 79^0 28' E and at the altitude of 426.7 m above mean sea-level **(Fig. 3).**

Fig 3 A general view of field experiment

2.2 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.

2.3 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 Azobtobacter, PSB, (Phosphate solubilizing bacteria) respectively. FYM (Farm yard manure) was applied in experimental plots just before sowing.

2.4 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.

2.5 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 Chaurasia and Chaurasia, 2024)**. The details of treatment as mentioned in **Table 1**.

Table 1 Description of Integrated nutrient management treatments.

Treatments	Details of treatment applications
T_1	100% NPK recommended dose 50:30:30 kg/ha
T_2	100% NPK/ha + 5 kg Zn/ha
T_3	10 tonnes FYM/ha
T_4	10 tonnes FYM/ha + 3 kg PSB/ha
T_5	10 tonnes FYM/ha + 3 kg Azotobacter/ha
T_6	10 tonnes FYM/ha + 3 kg Azotobacter/ha + 3 kg PSB/ha
T_7	10 tonnes FYM/ha + 3 kg Azotobacter/ha + 3 kg PSB/ha + 5 kg Zn/ha
T_8	50% NPK/ha + 5 tonnes FYM/ha
T_9	50% NPK/ha + 5 tonnes FYM/ha + 3 kg PSB/ha
T_{10}	50% NPK/ha + 5 tonnes FYM/ha + 3 kg Azotobacter/ha
T_{11}	50% NPK/ha + 5 tonnes FYM/ha + 3 kg Azotobacter/ha + 3 kg PSB/ha
T_{12}	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= Potassioum Zn= Zinc, FYM= Farm yard manure. PSB = Phosphate solubilizing bacteria, Kg = Kilogram and ha = Hectare.

2.6 Estimation of total withanolides, withaferin A, withanolide A and withanolide B content in Ashwagandha :

2.6.1 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 offical agriculture chemists, 1960)**, followed by repeated washed off with distilled water.

2.6.2 Reagents and standards :

All chemicals and solvents used analytical grade or HPLC grade and obtained from E-merck and ther renowned companies. Standards sample of withaferine A, withanolide A and withanolide B were procured from Natural Remedies Private Limited, Bangalore, India.

2.6.3 Collection of plant samples :

The treatment and replication wise mature root, stem and leaf 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 course particles and was used for the extraction of plant samples.

2.6.4 Extraction of plant samples :

One gram each of the powdered sample was extracted with 10 ml methanol under sonication for 30 minutes. The slurry was then centrifuged at 8000 rpm for 15 minutes. The supernatant was concentrated in rotary evaporator at 50 ^{0}C for 30 minutes. The remaining dried residue was re-dissolved in 5 ml methanol and centrifuged at 8000 rpm for 15 minutes. The supernatant was then filtered through 0.45 micron filter paper. The extract of each of the plant sample was used for the estimation of the content of total withanolides, withaferin A, withanolide A and withanolide B.

2.6.5 Estimation of total withanolides content:

A modified spectrophotometric metod **(Mishra, 1994)** was used to estimate total withanolides content in root, stem and leaf of Ashwagandha. For this purpose 1 ml plant extract was used for the development of colour by adding 1 ml chloroform and 4.0 ml Lieberman-Burchard reagent (L.B. reagent). After keeping for 5 minutes in an ice both, the optical density was recorded in a spectrophotometer at 530 nm. The parcentage of total withanolides content was calculated using cholesterol as standard.

2.6.6 Estimation of withaferin A, withanolide A and withanolide B Content :

For quantitative estimation of withaferin A, withanolide A and withanolide B content in root of Ashwagandha, the analysis was carried out by high performance liquid chromatography (HPLC) method (**Agarwal and Murali, 2010**).

Quantitative determination of the peak, the area value of the standard withaferin A, withanolide A and withanolide B with known concentration was compared with the plant sample peak and the percentage of withaferin A, withanolide A and withanolide B were calculated accordingly :

withaferin A(%), withanolide A(%) or withanolide B(%) =

$$\frac{\text{Area of the sampe}\times\text{Standard weight (mg)}\times\text{Sample dilution}\times\text{Purity of standard}}{\text{Area of the standard}\times\text{Sample weight (mg)}\times\text{Standard dilution}\times 100}\times 100$$

3. STATISTICAL ANALYSIS :

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

4. RESULTS AND DISCUSSION :

The mdicinal value of Ashwagandha (*Withania somnifera* (L.)Dunal) is not only assessed by its yield but also by the quality of the produce. The quality of Ashwagandha is often determined on the basis of bioactive plant metabolites mainly withanolides content. Though these characters are generally considered as varietal, however it has also been reported that it is influenced by the integrated nutrient management system.

4.1 Effect of integrated nutrient management system on total withanolides content in root, stem and leaf of Ashwagandha :

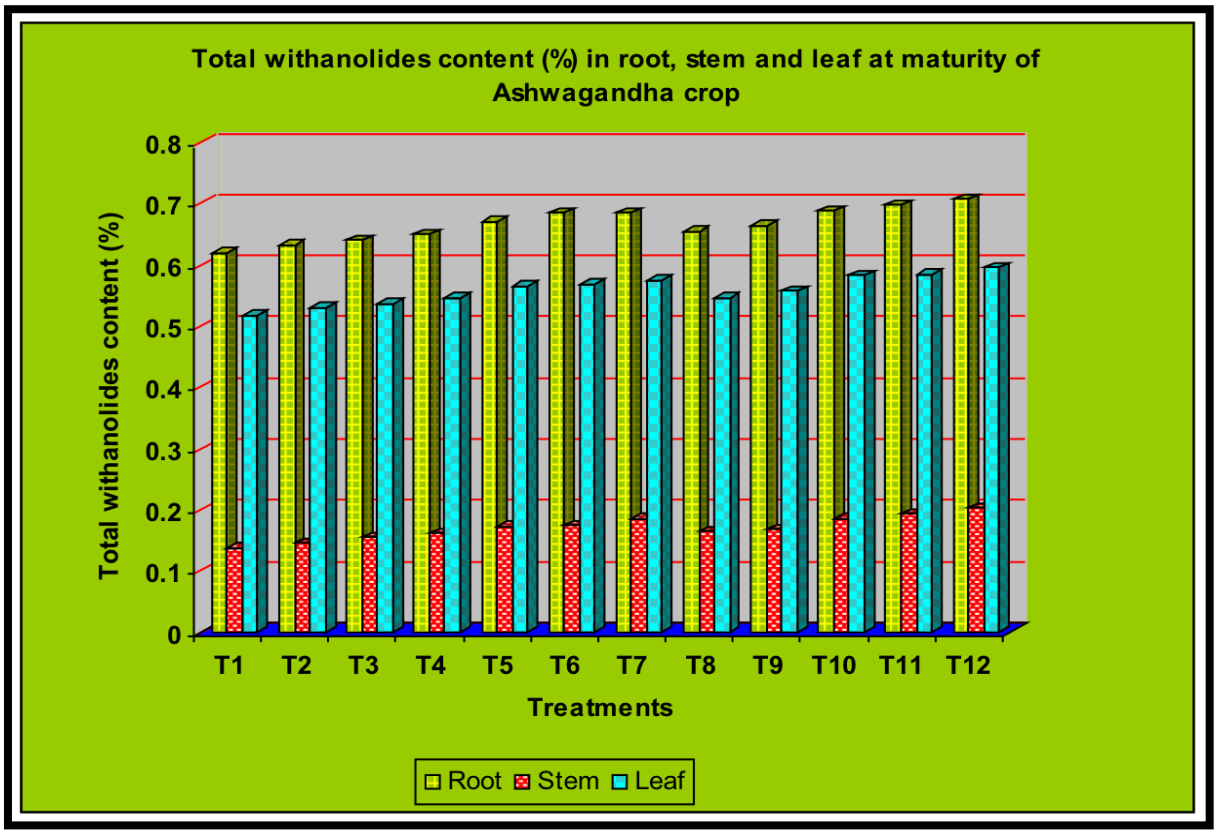
The total withanolides content in root, stem and leaf of Ashwagandha was studied under the influence of integrated nutrient management system are presented in **Table 2 and Fig. 3.** The statistically analyzed data indicated that total withanolides content in different plant parts of Ashwagandha was significantly influenced by integrated nutrient management system.

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

Treatments	Total withanolides content (%)		
	Root	Stem	Leaf
T1	0.618	0.136	0.516
T2	0.632	0.144	0.530
T3	0.639	0.154	0.536
T4	0.649	0.161	0.546
T5	0.669	0.171	0.564

T6	0.684	0.174	0.567
T7	0.684	0.185	0.574
T8	0.653	0.164	0.546
T9	0.663	0.167	0.557
T10	0.687	0.185	0.582
T11	0.697	0.192	0.584
T12	0.707	0.202	0.595
SEm±	0.003	0.002	0.002
CD (P=0.05)	0.008	0.006	0.007

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



Highest total withanolides content in root (0.707%) of Ashwagandha was recorded with the treatment of 50% NPK/ha + 5 tonnes FYM/ha + 3kg Azotobacter/ha + 3 kg PSB/ha + 5 kg Zn/ha (T_{12}) followed by 50% NPK/ha + 5 tonnes FYM/ha + 3kg Azotobacter/ha + 3kg PSB/ha (T_{11}) (0.697%). The lowest total withanolides content in root (0.618%) of Ashwagandha was recorded under the treatment of 100% NPK recommended dose 50:30:30 kg/ha (T_1).

Significantly maximum total withanolides content in stem (0.202%) of Ashwagandha was obtained by the treatment of 50% NPK/ha+5tonnes FYM/ha+3kg Azotobacter/ha + 3kg PSB/ha + 5kg Zn/ha (T_{12}) followed by 50% NPK/ha+ 5 tonnes FYM/ha + 3 kg Azotobacter/ha + 3kg PSB/ha (T_{11}) (0.192%). Significntly minimum total withanolides content in stem (0.136%) of Ashwagandha was noted in 100% NPK recommended dose 50:30:30 kg/ha (T_1).

Significantly maximum total withanolides content in leaf (0.595%) of Ashwagandha was noted in treatment 50% NPK/ha + 5 tonnes FYM/ha + 3kg Azotobacter/ha + 3kg PSB/ha + 5 kg Zn/ha (T_{12}) which was found at par with 50% NPK/ha +5 tonnes FYM/ha+ 3kg Azotobacter/ha + 3 kg PSB/ha (T_{11}) (0.584%). Significantly minimum total withanolides content in leaf (0.516%) of Ashwagandha was noted in 100% NPK recommended does 50:30:30 kg/ha (T_1).

The highest values of total withanolides content in root, stem and leaf of Ashwagandha may be due to the combined application of organic manure, biofertilizers and zinc might have supplied adequate amounts of nutrients which favoured higher metabolic rate and enzyme activities in the plant, resulting in higher percentage of total withanolides content. This in in accordance with the findings of **Patidar et al. (2014), Chaudhary et al. (2017), Malviya et al. (2017) and Basak et al. (2020)** in Ashwagandha. **Patidar et al. (2014)** reported that the total withanolides content in root, stem and leaf of Ashwagandha was recorded significantly maximum with treatment of 50% NPK recommended dose+ 5 tonnes FYM+ PSB+ Azotobacter+ 5 kg Zn/ha. **Chaudhary et al. (2017)** reported that the highest total withanolides content was recorded with application of castor cake which was at per with vermicompost. **Malviya et al. (2017)** reported that significantly higher values of total withanolides content in root, stem and leaf were observed with treatment 75% NPK recommended dose+ 5 tonnes vermicompost/ha + PSB+ 3 kg Azotobacter/ha. **Basak et al. (2020)** reported that the highest total withanolide contents were recorded under the treatment receiving castor cake+ microbial consortium followed by application of vermicompost + microbial consortium.

4.2 Effect of integrated nutrient management system on withaferin A, withanolide A and withanolide B content in root of Ashwagandha :

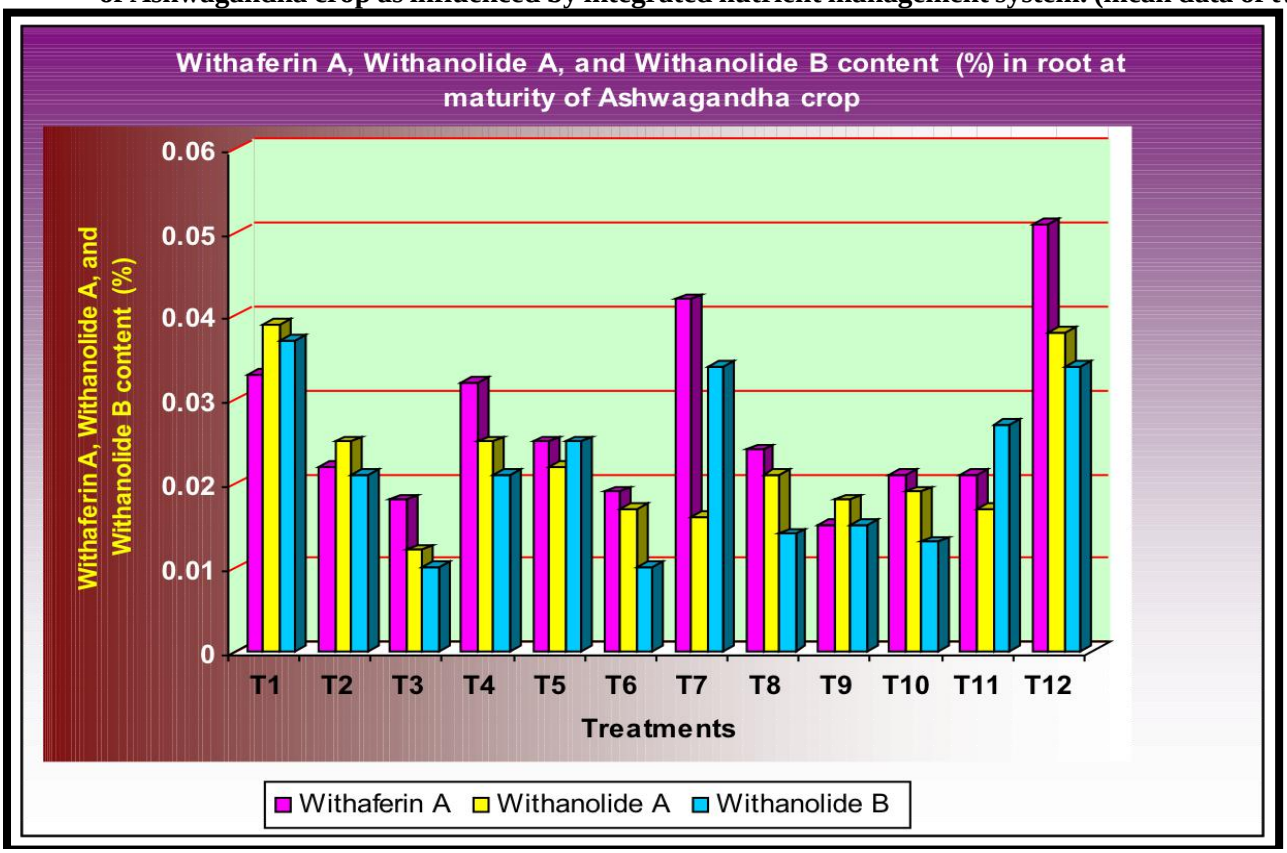
The pooled analysis of two years data recorded in **Table 3 and Fig. 4** explicit that withaferin A, withanolide A and withanolide B content in root of Ashwagandha were significantly affected by the integrated nutrient management system.

It is ovious from the data that 50% NPK/ha + 5 tonnes FYM/ha + 3kg Azotobacter/ha +3 kg PSB/ha + 5 kg Zn/ha (T_{12}) treatment signifficantly increased the withaferin A contnet in root of Ashwagandha and registering highest value (0.051%) followed by 10 tonnes FYM/ha +3kg Azotobacter/ha + 3kg PSB/ha + 5kg Zn/ha (T_7) (0.042%). The minimum withaferin A content (0.015%) in root of Ashwagandha was recorded under the treatment of 50% NPK/ha + 5 tonnes FYM/ha + 3kg PSB/ha (T_9).

Table.3 Effect of integrated nutrient management system on Withaferin A, Withanolide A, and Withanolide B content in root (%) at maturity of Ashwagandha crop (Mean data of two years)

Treatment	Withanolides content (%)		
	Withaferin A	Withanolide A	Withanolide B
T_1	0.033	0.039	0.037
T_2	0.022	0.025	0.021
T_3	0.018	0.012	0.010
T_4	0.032	0.025	0.021
T_5	0.025	0.022	0.025
T_6	0.019	0.017	0.010
T_7	0.042	0.016	0.034
T_8	0.024	0.021	0.014
T_9	0.015	0.018	0.015
T_{10}	0.021	0.019	0.013
T_{11}	0.021	0.017	0.027
T_{12}	0.051	0.038	0.034
SEm±	0.002	0.001	0.001
CD (P=0.05)	0.005	0.002	0.002

Fig.4 : Different withanolides content (Withaferin A, Withanolide A, and Withanolide B) in root (%) at maturity of Ashwagandha crop as influenced by integrated nutrient management system. (mean data of two years)



Significantly maximum withanolide A content (0.039%) in root of Ashwagandha was noted in treatment of 100% NPK recommended dose 50:30:30 kg/ha (T_1) which was found at par with 50% NPK/ha +5 tonnes FYM/ha+ 3kg Azotobacter/ha+ 3kg PSB/ha + 5 kg Zn/ha (T_{12}) (0.038%). Significantly minimum withanolide A content (0.012%) in root of Ashwagandha was noted in treatment of 10 tonnes FYM/ha (T_3).

Signifficantly maximum withanolide B content (0.037%) in root of Ashwagandha was obtained by the treatment of 100% NPK recommended dose 50:30:30 kg/ha which was at par with 10 tonnes FYM/ha+ 3kg Azotobacter/ha + 3 kg PSB+ha + 5kg Zn/ha (T_7) (0.034%) and 50% NPK/ha + 5 tonnes FYM/ha + 3 kg Azotobacter/ha + 3 kg PSB/ha + 5 kg Zn/ha (T_{12}) (0.034%). Significantly minimum withanolide B content (0.010%) in root of Ashwagandha was noted in treatment of 10 tonnes FYM.ha (T_3) and 10 tonnes FYM/ha + 3kg Azotobacter/ha + 3kg PSB/ha (T_6).

Withaferin A content in root mostly influence by those treatments which have involment of organic manure, biofertilizers and optimum amount of Zinc. organic manure, phosphate solubilizing bacteria, Azotobacter along with zinc not only involves the physical status of soil, but also increases the organic status of the soil, resulting in rapid multiplication of beneficial soil microbes, there by promotic the availability and uptake of nutrients by the plants which favoured higher metabolic rate and enzyme activities in plant resulting in increase withaferin A content in root of Ashwagandha.

The application of inorganic fertilizers migh have supplied adequate amount of nutrients which fovoured higher metabolic rate and enzyme activities in plant resulting in higher withanolide A and withanolide B content in root of Ashwagandha. The more or less similar results are observed by **Shrivastava et al. (2012), Yadav et al. (2013). and Ramanuj and Shelet (2018)** in Ashwagandha**. Shrivastav et al. (2012**) reported that application of NPK 40:20:20 kg/ha along with 2.5 tonnes vermicompost/ha and 5 tonnes FYM/ha + 20 kg Zn/ha produced maximum withaferin A content in *Withania somnifera* roots. **Yadav et al. (2013)** found that withaferin A was maximum with the application of 50% NPK/ha + 5 tonnes FYM+PSB+Azotobacter+ 5kg Zn/ha. **Ramanuj and Shelet (2018)** reported that soil application of *Pseudomonas aeruginosa* (1 l/ha) along with RDF (30:15:O NPK Kg/ha) produced maximum withaferine A content in *Withania somnifera* roots.

CONCLUSION :

From the findings of present investigation it could be concluded that total withanolides, withaferin A, withanolide A and withanolide B content in Ashwagandha were significantly influenced by integrated nutrient management system. The maximum values of total withanolides content in root, stem, leaf and withaferin A content in root of Ashwagandha were recorded with the application of 50% NPK/ha +5 tonnes FYM/ha+ 3kg Azotobacter/ha+ 3kg PSB/ha + 5 kg Zn/ha (T_{12}). Where as, maximum values of withanolide A and withanolide B content in root of Ashwagandha were recorded with the application of 100% NPK recommended dose 50:30:30 kg/ha (T_1). Based on overall expermental results, it could be concluded that 50% NPK/ha+5tonnes FYM/ha+3 kg Azotobacter/ha+ 3kg PSB/ha + 5kg Zn/ha (T_{12}) was found to be better inegrated nutrient management system for better synthesis and accumulation of total withanolides and withaferin A content where as, better synthesis and accumulation of withanolide A and withanolied B content was found in the treatment of 100% NPK recommended dose 50:30:30 kg/ha (T_1).

REFERENCES :

1. **Ahmad, M. and Dar, N.J. (2017).** "*Withania Somnifera* : Ethnobotany, Pharmacology and Therapeutic function" *in sustained energy for enhanced human functions and activity (Academic Press) pp 137-154.*
2. **Behl, T., Sharma, A., Sharma, L., Sehgal, A., Zengin, G., Brata, R., Fratila, O. and Bungau, S., (2020).** Exploring the multifaced therapeutic potential of withaferin A and its derivatives. *Biomedicine. 8 (12) : 571.*
3. **Chaurasia, Sushmita and Singh, Rashmi (2022).** Effect of integrated nutrient management system on yield attributes, yield and economics of Ashwagandha (*Withania somnifera* (L.) Dunal). *International Journal of Current Science. 12(2) : 796-815.*
4. **Paul, S., Chakraborty, S., Anand, U., Dey, S., Nanoly, S., Ghorai, M., Saha, S.C., Patil, M.T., Kandimalla, R., Prockow, J. and Dev, A. (2021).** *Withania sominfera* (L.) Dunal (Ashwagandha) : A comprehensive review on ethnopharmacology, pharmacotherapeutics, biomedicinal and toxicological aspect, *Biomed Pharmacother.* 143, 112175.
5. **Uddin, Q., Samiulla, L., Singh, V.K. and Jamil, S.S. (2012).** Phyto chemical and pharmacological profile of *Withania somnifera* Dunal : A Review. *Journal of Applied pharmaceutical Science. 2 (1) : 170-175.*
6. **Pandian, A., Kaliyaperumal, A.K., Sekar, S., Sivakumar, P., Vijai Selvaraj, K.S., Karthik, M. and Hariprasath, L. (2020)**. Botany and ethnopharmacological potential of Ashwagandha. *Journal of Current Opinion in Crop Science. 1 (1) : 35-40.*
7. **Saleem, S., Muhammad, G., Hussain, M.A., Altaf, M. and Bukhari, S.N.A. (2020).** *Withania somnifera* L. insights into the phytochemical profile, therapeutic potential clinical trials and future prospective. Iran J. Basic Med. Sci. 23 (12) : 1501-1526.
8. **Chaurasia, Sushmita and Chaurasia, Shubha (2024).** Effect of integrated nutrient management system on total alkaloid content in different plant parts of Ashwagandha (*Withania somnifera* (L.) Dunal). *Journal of Emerging Technologies and Innovative Research. 11 (1) : 566-577.*
9. **Ku, S.K., Han, M.S. and Bae, J.S. (2014).** Withaferin A is an inhibitor of endothelial protein C receptor shedding in vitro and invivo. Food Chem. Texicol 68 : 23-29.
10. **Kumar, V., Dey, A., Hadimani, M.B., Marcovic, T. and Emerald, M. (2015).** Chemistry and pharmacology of *Withania somnifera* : Anupdate CELLMED. 5 (1) : 11-113.
11. **Parihar, S. (2022)**. A literature review on pharmacological activities of *Withania somnifera* Biol. Sci. 2 (1) : 147-154.
12. **Trivedi, M.K., Panda, P., Sethi, K.K. and Jana, S. (2017).** Metabolite profiling in *Withania somnifera* roots hydroalcoholic extract using LC/MS, GC/MS and NMR spectroscopy. *Chem. Biodivers*. 14 (3) : e1600280.
13. **Chourasia, N.D., Uniyal, G.C., Payare lal, Mishra, L.N., sangwan, N.S., Tuli, R. and Sangwan, R.S. (2008).** Analysis of withanolides in root and leaf of *Withania somnifera* by HPLC with photodiode array and evaporative light scattering detection. *Photochemical Analysis. 19 : 148-154.*
14. **Patidar, J. Naruka, I.S., Shaktawat, R.P.S. and Verma, K.S. (2014).** Integrated nutrient management in Ashwagandha (*Withania somnifera* (L.) Dunal). *Environment and Ecology, 32 (4A) : 1465-1469.*
15. **Gajbhiye, N., Makasana, J. and Kumar, S. (2015).** Accumulation of three important bioactive compounds in different plant parts of *Withania somnifera* and its determination by the LC-ESI-MS-MS (MRM) method. *J. Chromatogr. Sci. pp. 1-8.*

16. **Malviya, N., Naruka, I.S., Gallani, R., Singh, O.P. and Patidar, D.K. (2017).** Effect of integrated nutrient managment on growth, yield and quality of Ashwagandha (*Withania somnifera* (L.) Dunal). *Environment and Ecology. 35 (4B) : 3206-3210.*
17. **Singh, M., Bhutani, S., Dinkar, N., Mishra, A., Perveen, K., Alfagham, A.T., Khanam, M.N., Bhatt, S.C., and Suyal, D.C. (2023).** Estimating the production of withaferin A and withanolide A in *Withania somnifera* (L.) Dunal using aquaponics for sustainable development in hill agriculture. Frontiers in plant Science. 14 : 1215592.
18. **Sangwan, R.S., Chaurasia, N.D., Lal, P., Misra, L., Tuli, R., and Sangwan, N.S. (2008).** Withanolide A is inherently de nova biosynthesized in roots of the medicinal plant Ashwagandha (*Withania somnifera*). *Physiologia Plantarum. 133 (2) : 278-287.*
19. **Chatterjee, S., Shrivastava, S., Khalid, A., Singh, N., Sangwan, R., Sidhu, O.P., Roy, R., Khetrapal, C.L., and Tuli, R. (2010).** Comprehensive metabolic fingerprinting of *Withania sominifera* leaf and root extracts. *Phtochemistry.71 (10) : 1085-1094.*
20. **Mengistu, D.K. and Mekonnen, L.S. (2012).** Integrated agronomic crop managements to improve of productivity under terminal improved of productivity under terminal drought. Water stress, In tech open. pp 235-254.
21. **Suba Rao, N.S. (1982.** Biofertilizers in Agriculture. Oxford and IBJ Pub. Co. New Delhi.
22. **Mady, M.A. and Youssef, A.S.M. (2014).** Influence of some fartilizers and boron foliar spray on improving growth and oil productivity of dragonhead (*Dracocephalum moldavica* L.) *Plant J. production*. 5 (4) : 587-613.
23. **Alexander, M. (1977).** Introduction to soil microbiology. 2nd Edn, John Wiely and Sons, Newyour. pp 233.
24. **Chaurasia, Sushmita and Singh, Rashmi (2021a).** Influence of integrated nutrient management system on phenological and growth attributing characters of Ashwagandha (W*ithania somnifera* L. Dunal). *International Journal of Current Research. 13 (09) : 18967-18972.*
25. **Chaurasia, Sushmita and Singh, Rashmi (2021b).** Effect of integrated nutrient management system on chlorophyll a, chlorophyll b and carotenoids content of Ashwagandha (*Withania somnifera* (L.) Dunal). *International Journal of Advanced Research in Biological Sciences. 8 (10) : 154-165.*
26. **Association of official agricultural chemists (1960).** *Official method 9th Edn, Association office Agric., Chem., Washington.*
27. **Mishra, S.N. (1994).** Colorimetric method for estimation of total withanolide. 10th All India Workshop Report on Medicinal and Aromatic plants. 379-381.
28. **Agarwal, A. and Murali, B. (2010).** Estimation of withanolides in Ashwagandha by HPLC. *Quality assessment of selected Indian medicinal plants. pp 241-243.*
29. **Fisher, R.A. and Tates, F. (1963).** Statistical tables Oliver and Boyd, Edinburgh Tweeddale court, London.
30. **Fisher, R.A. (1967).** Statistical methods of research workers. *13" Edn. Hafner Publishing Edinburgh.*
31. **Chaudhary, S.R., Jitendra Kumar., Chaudhary, A.P. and Basak, B.B. (2017a).** Organic nutrient sources influence yield and quality of Ashwagandha *(Withania somnifera),* and soil biochemical properties. *Agrochimica. 61 (3) : 221-236.*
32. **Basak, B.B., Saha, A., Gajbhiye, N.A. and manivel, P. (2020).** Potential of organic nutrient sources for improving yield and bioactive principle of Ashwagandha (*Withania somnifera*) through enhanced soil fertility and biological functions. *Communications in Soil Science and Plant Analysis. 51 (6) : 779-793.*
33. **Shrivastava, A., Verma, K., Kewat, M.L. and Nema, J. (2012).** Effect of integrated nutrient management on quality content of Ashwagandha. *Journal of Medicinal and Aromatic Plant Sciences, 34 (1&2) : 67-70.*
34. **Yadav, M.S., Yadav, B and Singh, S. (2013).** Response of Ashwagandha (*Withania somnifera* (L.) Dunal) to integrated nutrient management system (INMS). *Crop Rsearch. 45(1-3): 276-279.*
35. **Ramanuj, K.B. and Shelat, H.N. (2018).** Enhancement of yield and quality parameters of *Withania somnifera* by indigenous endophytic bacterial isolates. *Int. J. Curr. Microbiol. App. Sci. 7 (2) : 2569-2581.*