



Effect of Integrated Nutrient Management System on Chlorophyll a, Chlorophyll b and Carotenoids content of Ashwagandha (*Withania somnifera* L.Dunal)

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Abstract

The Integrated nutrient management system is important for crop productivity and soil health. Ashwagandha (*Withania somnifera* L. Dunal) is one of the most important herbs of Ayurveda (the traditional system medicine in India) used for millennia as a Rasayana for its wide-ranging health benefits. It is commonly known as “Indian Winter cherry” or “Indian Ginseng”.

A field experiment was conducted during the Kharif season of 2017-18 and 2018-19 near the college of agriculture farm, Tikamgarh (M.P.) India to study the effect of integrated nutrient management system on chlorophyll a, chlorophyll b and carotenoids content at different growth periods of Ashwagandha. The experiments was laid out in randomized block design with three replication and twelve treatments viz, 100% NPK recommended dose 50:30:30 kg/ha (T₁), 100% NPK/ha + 5 kg Zn/ha (T₂), 10 tonnes FYM/ha (T₃), 10 tonnes FYM /ha + 3 kg PSB/ha (T₄), 10 tonnes FYM/ha + 3 kg Azotobacter/ha (T₅), 10 tonnes FYM/ha + 3 kg Azotobacter/ha + 3kg PSB/ha (T₆), 10 tonnes FYM/ha + 3 kg Azotobacter/ha + 3 kg PSB/ha + 5 kg Zn/ha (T₇), 50% NPK/ha + 5 tonnes FYM/ha (T₈), 50% NPK/ha + 5 tonnes FYM/ha + 3 kg PSB/ha (T₉), 50% NPK/ha + 5 tonnes FYM/ha + 3 kg Azotobacter/ha (T₁₀), 50% NPK/ha + 5 tonnes FYM/ha + 3 kg Azotobacter/ha + 3 kg PSB/ha (T₁₁) and 50% NPK/ha + 5 tonnes FYM/ha + 3 kg Azotobacter/ha + 3 kg PSB/ha + 5 kg Zn/ha (T₁₂). The Chlorophyll a, chlorophyll b and carotenoids content of fresh leaves were recorded by spectrophotometry and results were expressed as milligram per gram of fresh leaf weight. The pooled analysis of two years' data indicated that an integrated nutrient management system significantly influenced chlorophyll a, chlorophyll b and carotenoids content of Ashwagandha. Integrated nutrient management system resulted in the highest chlorophyll a and chlorophyll b content was recorded at 90 DAS (days after sowing). However, carotenoids content was recorded at 75 DAS. The highest values of chlorophyll-a (2.17 mg/g fresh leaf weight), chlorophyll b (0.48 mg/g fresh leaf weight and carotenoids (0.49 mg/g fresh leaf weight) 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 (T₁₂ treatment). Based on overall experimental results, it could be concluded that 50% NPK/ha + 5 tonnes FYM/ha + 3 kg Azotobacter/ha + 3 kg PSB/ha + 5 kg Zn/ha (T₁₂ treatment) was found to be a better-integrated nutrient management system which can be used for better quality production of Ashwagandha (*Withania somnifera* L.Dunal.) Crop.

Keywords: Integrated nutrient management system, chlorophyll a, chlorophyll b, carotenoids and Ashwagandha (*Withania somnifera* L. Dunal.).

Introduction

Ashwagandha (*Withania somnifera* L.Dunal.) belonging to the family Solanaceae, is an Ayurvedic herb also known as Indian winter cherry and Indian ginseng that has been traditionally known since ancient times in India for its numerous beneficial health activities. Ashwagandha is one of the most important herbs in Ayurveda, which has been used for > 3000 years in stress management, energy elevation and improving cognitive health (Rege *et al.*, 1999; Singh *et al.*, 2008; Pratte *et al.*, 2014 and Farooqui *et*

al., 2018) and to lower inflammation, blood sugar levels, cortisol, anxiety and depression (Mirjalili *et al.*, 2009, and Montalvan *et al.*, 2015). The plant is an erect, grayish evergreen shrub with long tuberous roots, short stem, ovate and petiolate leaves and greenish axillary and bisexual flowers (Fig.- 1 A, B.) The perception of the colour of the plant is due to the presence of pigments. These pigments play important roles in plant metabolism and visual attraction in nature (Goodwin, 1976; 1988; Gross, 1987, 1991; Kost, 1988).



(A)

(B)

Fig. 1 A, B Showing the green leaves and flowers in Ashwagandha (*Withania somnifera* L. Dunal) plant

Chlorophyll a, chlorophyll b and carotenoids are very important pigments that have been used as intrinsic optical molecular probes to observe plant performance during different phases of development (Goodwin, 1988; Gross, 1991 and Kost, 1988). These pigments are biosynthesized in chloroplast and their metabolism is closely related to chloroplast development. In-plant the ratio of chlorophyll a to chlorophyll b is about three to one. Chlorophyll a (blue-green) and chlorophyll b (yellow-green) have very similar structures. Both are 'tadpole' shaped due to a hydrophobic tail and hydrophilic head. The head

consists of a porphyrin ring with magnesium (Mg) in the center. The porphyrin ring of chlorophyll is where light energy is absorbed. Chlorophyll a and chlorophyll b differ in only one atom in a side chain on the third carbon. In chlorophyll a, the third carbon is attached to a methyl group (-CH₃) whereas, in chlorophyll b, the third carbon is attached to an aldehyde group (-CHO). The structure of chlorophyll is shown in Fig.-2. Carotenoids (yellow to yellow-orange) molecules are isoprenoids that consist of eight isoprene units in a long polyene chain, which may extend from 3 to 15 conjugated double bonds (Fig. 3).

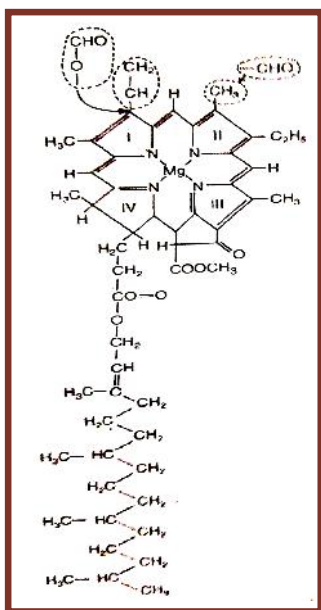


Fig. 2 Molecular structure of chlorophyll

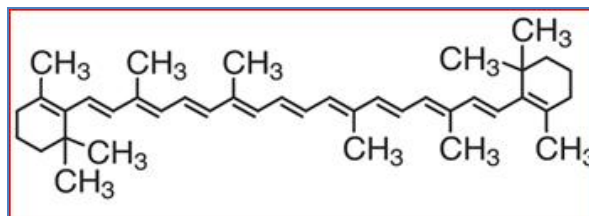


Fig. 3 Molecular structure of carotenoids (S carotene)

The function of chlorophyll a chlorophyll b and carotenoids are mainly light-harvesting, energy transfer, photochemical redox reaction as well as photoprotection. The primary pigment responsible for photosynthesis is chlorophyll a, and chlorophyll b absorbs light energy to pass it on to chlorophyll a. Being essential structural components of the photosynthetic antenna, carotenoids participate in the harvesting light energy for photosynthesis (Havaux *et al.*, 1998; Holt *et al.*, 2005 and Zakar *et al.*, 2016). In addition to the direct contribution in the photosynthetic process, carotenoids are also involved in the defense mechanism against oxidative stress (Boo and Jung, 1999; Bouvier, *et al.*, 2005; Campos, *et al.*, 2016) and play an essential role in the dissipation of excess light energy and provide protection to reaction centers (Demmig-Adams and Adam, 1996., 2006 Marin, 2011; Santabarbara *et al.*, 2013; Nagy *et al.*, 2015).

The accurate estimation of leaf photosynthetic pigments is an important element in monitoring fertilizer application and managing the overall vegetation health particularly in an agricultural system where productivity levels are directly related to plant condition (Shah *et al.*, 2017). Chlorophyll concentration in leaves is an indicator of plant health (Porra, 2002). The chlorophyll a:b ratio also indicates the development of higher plants. The leaf chlorophyll content provides a key indicator of the photosynthetic capacity (Kozłowski *et al.*, 1991) and in combination with measurements such as leaf area index is a critical

proxy for vegetation productivity (Gitelson, *et al.* 2006). Determination of chlorophyll content is an indirect method of estimating the productivity also provides a good understanding of the photosynthetic regime of plants (Bojovic and Stojanovic, 2005).

Nutrients supplied by fertilizers play a fundamental role in the structural and functional components of photosynthetic machinery (Schertz, 1928; Pearman *et al.*, 1979; Yong *et al.*, 2010) and an optimal nutrient supply is considered essential for the biosynthesis of plant photosynthetic pigments (Cai *et al.*, 2008; Hosseinzadeh *et al.*, 2016). Any deficiencies will likely lead to a reduced content of leaf pigments retarded plant growth and low net primary productivity (Zhang *et al.*, 2003).

The use of an integrated nutrient management system has assumed great importance for the biosynthesis of plant pigments, soil health, and plant nutrient supply at an optimum level for sustaining the desired productivity through optimization of the benefits from all possible sources of organic, inorganic and biological components in an integrated manner. Integrated use of inorganic fertilizers, organic manure and biofertilizers is the only alternative that may help in enhancing plant photosynthetic pigments and sustained productivity. Several studies showed integrated nutrient management system significantly enhanced photosynthetic pigments of various plant species (Acharya, 2003; Najm *et al.*, 2012; Jaisankar, 2014; Priya, *et al.*, 2014; Chopra *et al.*, 2017).

Given above, the present study was carried out to investigate the effect of integrated nutrient management system on chlorophyll a, chlorophyll b and carotenoids content at different growth periods of Ashwagandha (*Withania somnifera* L.Dunal.) Crop.

Materials and Methods

The precise description of materials used and methodology adopted in the field and laboratory during experimentation is presented under the following appropriate heads.

Experimental site:

A field experiment was conducted during the Kharif season of 2017-18 and 2018-19 near the college of agriculture farm, Tikamgarh, Madhya Pradesh, India. The experimental site lies between the latitude 24° 26' N to 25° 40' N and longitude 78° 26' E to 79° 28' E at the altitude of 426.7 m above mean sea level. The region received annual rainfall ranging from 800 to 1000 mm and most of which was received from the end of June to the end of September.

Experimental field soil:

Soil analysis revealed that the experimental field soil was clay loam in texture slightly alkaline in reaction having pH 7.4 to 7.6, electrical conductivity 0.33 to 0.37 dS/m at 25°C and low organic carbon 0.43 to 0.45%. Soil was poor in available nitrogen 189 to 191 kg/ha, phosphorus 17.9 to 18.1 kg/ha, sulphur 15.5 to 16.5 kg/ha, high in available potash 298 to 302 kg/ha, low in DTPA-Fe 6.6 to 6.8 mg/kg, DTPA-Zn 0.37 to 0.41 mg/kg, DTPA-Cu 0.29-0.31 mg/kg and DTPA-Mn 2.7 to 3.1 mg/kg.

Sowing of the crop:

The seeds of Ashwagandha (JA-20) were sown in each plot at the rate of 10 kg/ha by hand at depth of 5 cm. in open furrows.

Treatment details:

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

Table – 1 Description of integrated nutrient management treatments

Treatments	Details of treatment application
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 = Potassium, Zn = Zinc, FYM = Farm yard manure, PSB = Phosphate solubilizing bacteria, kg = Kilogram and ha = Hectare.

Determination of chlorophyll a, Chlorophyll b and carotenoids content:

Glass Ware and distilled water:

Corning glassware and double distilled water were used throughout the present experimentation.

To avoid the decomposition of chlorophyll a, chlorophyll b and carotenoids pigments, 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 leaf samples:

Fresh Leaves of Ashwagandha (*Withania somnifera* L.Dunal.) from each five sample plants per plot were collected at 60, 75, 90, 105 and 120 DAS (days after sowing) and brought to the laboratory in separate polythene bags, lined with a moist filter paper inside. Care was taken to avoid the excessive loss of moisture from the leaf by preserving them in distilled water.

Extraction of chlorophyll a, chlorophyll b and carotenoids content:

The chlorophyll a, chlorophyll b and carotenoids content of leaves were extracted by acetone extraction method as per the procedure described by Yoshida *et al.*, 1972.

250 mg of the fresh leaf of each sample was finally cut and gently mixed with a clean pestle and mortar. To this homogenized leaf material, 10 ml of 80% acetone was added. The leaf materials were further ground gently. The sample was then put into a refrigerator at 4°C for 4 hours, thereafter the sample was centrifuged at 10000 rpm for 10 minutes. The supernatant was transferred to a 100 ml volumetric flask. The final volume was made up to 25 ml with the addition of 80% acetone. The optical densities of the solution were recorded by spectrophotometer at 480, 510, 645 and 663 nm wavelength against the solvent acetone (80%) was used as a blank (Sadasivam and Manickam, 1996). The amount of chlorophyll a, chlorophyll b and carotenoids content were calculated in terms of a milligram per gram (mg/g) fresh leaf weight.

Estimation of chlorophyll-a chlorophyll b and carotenoids content:

The amount of chlorophyll a, chlorophyll b and carotenoids were calculated by using the following formula (Duxbury and Yentach, 1956 and Maclachalam and Zalik, 1963).

Chlorophyll a (mg/g fresh leaf weight) =

$$\frac{12.7 \times (A_{663}) - 2.69 \times (A_{645})}{a \times 1000 \times W} \times V$$

Chlorophyll b (mg/g fresh leaf weight) =

$$\frac{22.9 \times (A_{645}) - 4.68 \times (A_{663})}{a \times 1000 \times W} \times V$$

Carotenoids (mg/g fresh leaf weight) =

$$\frac{7.6 \times (A_{480}) - 1.49 \times (A_{510})}{a \times 1000 \times W} \times V$$

Where,

- A = Optical density
- a = Length of light path (1 cm.)
- W = Fresh leaf weight (0.25g)
- V = Volume of Solution (25 ml)

Results and Discussion

Effect of integrated nutrient management system on chlorophyll a and chlorophyll b content:

The pooled analysis of two years' data indicated that the chlorophyll a and chlorophyll b content of Ashwagandha at 60, 75, 90, 105 and 120 DAS (days after sowing) were significantly affected by the integrated nutrient management system as presented in Table-2, Graph-1, Table-3 and Graph-2.

Table- 2 Effect of integrated nutrient management system on chlorophyll a content (mg/g fresh leaf weight) at different growth stages of Ashwagandha (Pooled for two years)

Treatment	Chlorophyll a content (mg/g fresh leaf weight)				
	Days after sowing				
	60 DAS	75 DAS	90 DAS	105 DAS	120 DAS
T ₁	1.34	1.41	1.56	1.43	1.37
T ₂	1.35	1.42	1.56	1.45	1.39
T ₃	1.34	1.40	1.53	1.44	1.37
T ₄	1.34	1.40	1.54	1.45	1.37
T ₅	1.45	1.52	1.86	1.55	1.49
T ₆	1.46	1.53	1.95	1.56	1.50
T ₇	1.53	1.61	2.15	1.63	1.57
T ₈	1.36	1.43	1.94	1.45	1.40
T ₉	1.45	1.53	1.60	1.55	1.49
T ₁₀	1.43	1.50	1.79	1.53	1.46
T ₁₁	1.46	1.54	2.01	1.56	1.50
T ₁₂	1.54	1.62	2.17	1.65	1.58
SEm ±	0.006	0.006	0.019	0.006	0.006
CD (P=0.05)	0.018	0.019	0.056	0.019	0.019

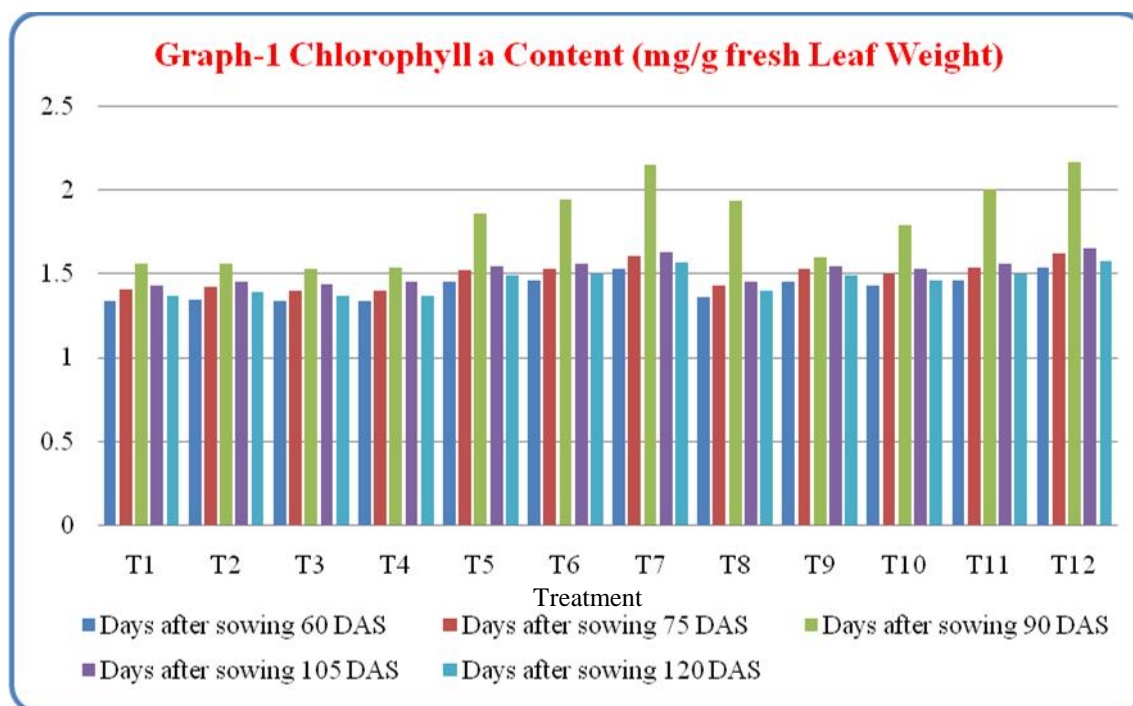
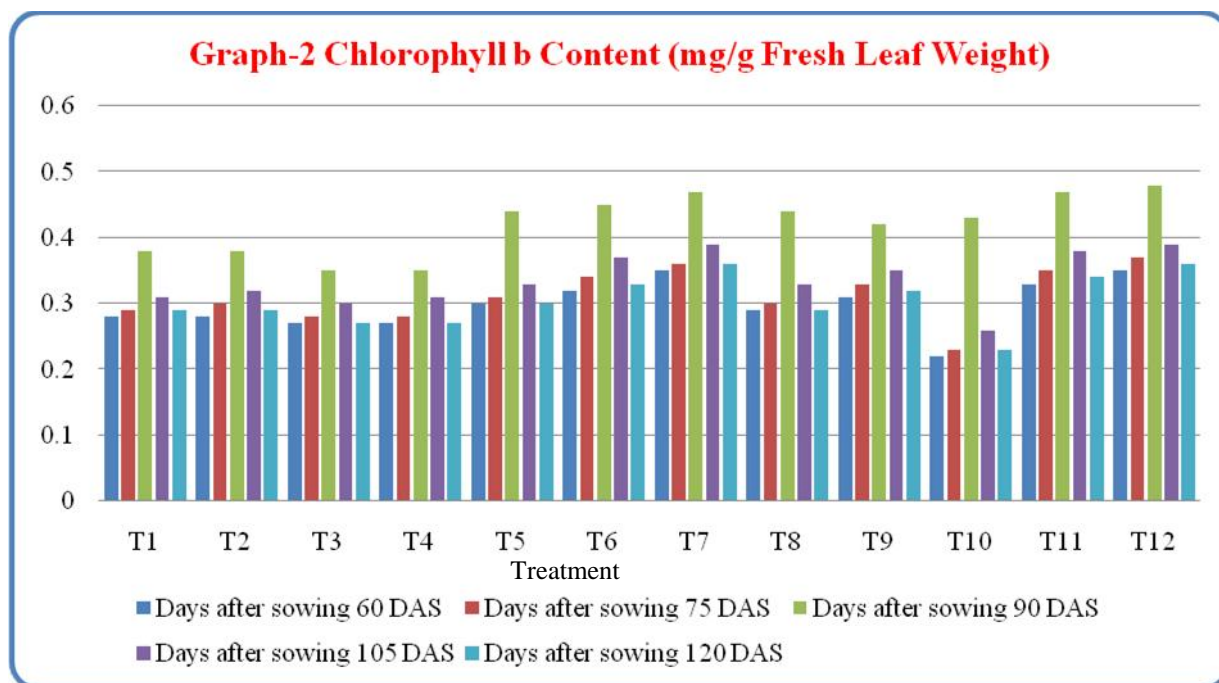


Table- 3 Effect of integrated nutrient management system on chlorophyll b content (mg/g fresh leaf weight) at different growth stages of Ashwagandha (Pooled for two years)

Treatment	Chlorophyll b content (mg/g fresh leaf weight)				
	Days after sowing				
	60 DAS	75 DAS	90 DAS	105 DAS	120 DAS
T ₁	0.28	0.29	0.38	0.31	0.29
T ₂	0.28	0.30	0.38	0.32	0.29
T ₃	0.27	0.28	0.35	0.30	0.27
T ₄	0.27	0.28	0.35	0.31	0.27
T ₅	0.30	0.31	0.44	0.33	0.30
T ₆	0.32	0.34	0.45	0.37	0.33
T ₇	0.35	0.36	0.47	0.39	0.36
T ₈	0.29	0.30	0.44	0.33	0.29
T ₉	0.31	0.33	0.42	0.35	0.32
T ₁₀	0.22	0.23	0.43	0.26	0.23
T ₁₁	0.33	0.35	0.47	0.38	0.34
T ₁₂	0.35	0.37	0.48	0.39	0.36
SEm ±	0.002	0.002	0.003	0.002	0.002
CD (P=0.05)	0.006	0.007	0.009	0.007	0.006



The values of chlorophyll a and chlorophyll b content were increased rapidly in almost all the twelve integrated nutrient treatments with the advancement in age of plant up to 90 DAS, thereafter decreased sharply up to 120 DAS of observation. It means that 90 DAS of plant growth is the best period for the maximum synthesis of chlorophyll a and chlorophyll b content. The present study suggests that the synthesis of chlorophyll a and chlorophyll b was optimum at a

definite period of plant growth, thereafter decrease towards maturity. The reason for this may be due to the decrease of nutrients supply to the plants or leaf senescence or due to the activity of chlorophyll degradation enzyme of plant tissues (Upadhey and Karadge, 1991; Kaewsuksaeng, 2011; Indrasti *et al.*, (2018). Recently Indrasti *et al.* (2018) mentioned the following pathway of chlorophyll degradation (Fig. 4) by chlorophyll degradation enzyme (chlorophyllase):

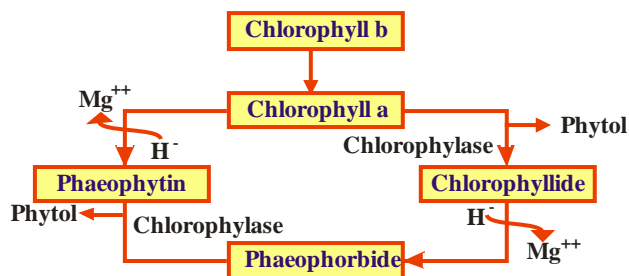


Fig- 4 General Pathway of chlorophyll degradation (Indrasti *et al.*, 2018)

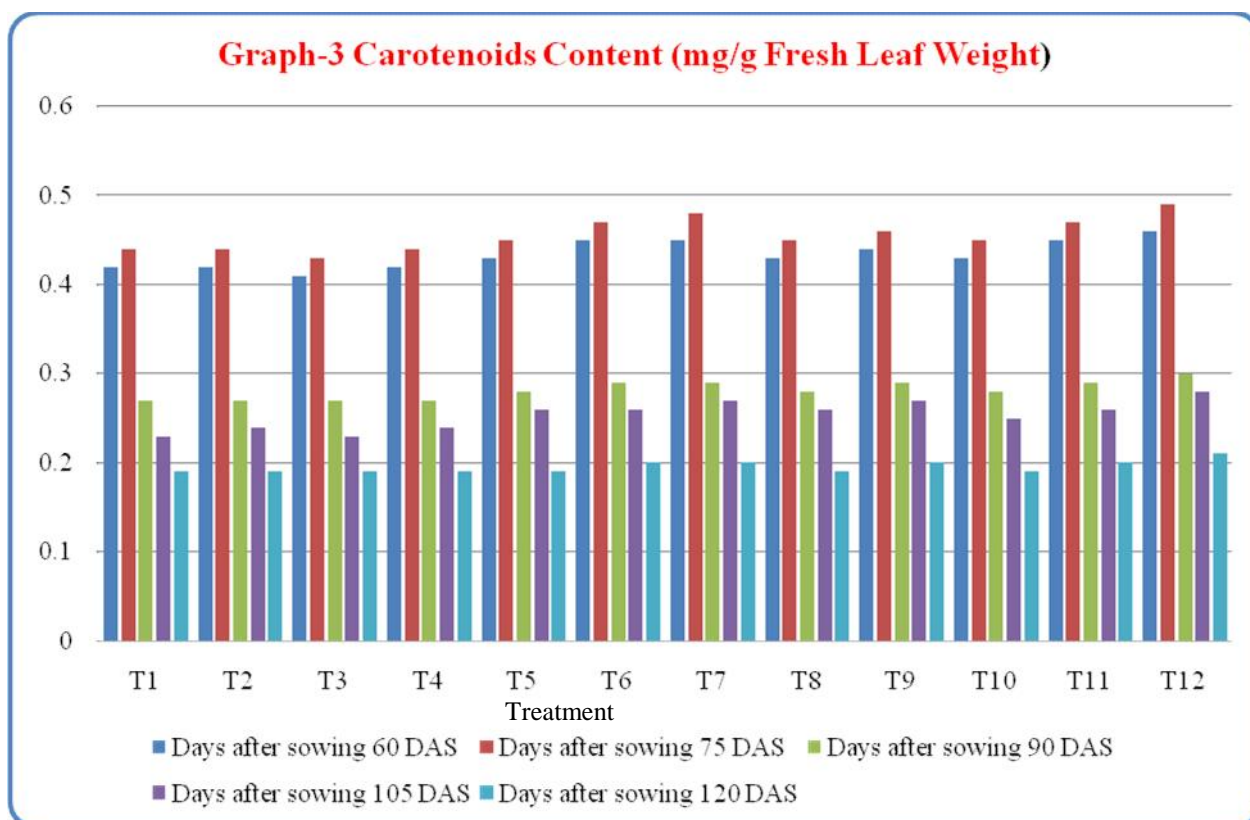
Amongst the integrated nutrient treatments the application of 50% NPK/ha + 5 tonnes FYM/ha + 3 kg Azotobactor/ha + 3 kg PSB/ha + 5 kg Zn/ha (T₁₂ treatment) was found to be most suitable in which highest values of chlorophyll-a (2.17 mg/g fresh leaf weight) and chlorophyll b (0.48 mg/g fresh leaf weight) were recorded. This may be due to a higher supply of nutrients especially nitrogen from inorganic, organic and biofertilizers sources and hence increased chlorophyll a and chlorophyll b content. The present findings were following Laha *et al.*, (1996); Acharya, (2003); Najm *et al.*, (2012); Priya *et al.*, (2014). Laha *et al.* (1996) reported that the application of N, P and K fertilizers to *Anacardium occidentale*, significantly affected the chlorophyll b content of leaves. Acharya (2003) reported that biofertilizers along with other organic and inorganic fertilizers could increase chlorophyll content in *Bambusa bambos*. Najam *et al.* (2012) reported that chlorophyll a, chlorophyll b and total chlorophyll content of *Agria potato* leaves were increased linearly and vary significantly with the application of nitrogen fertilizers and manure, Priya *et al.* (2014) reported that the highest chlorophyll content in *Zea mays* was obtained with application of 50% NPK which was at par with 100% NPK + 10 tone FYM per hectare.

Effect of integrated nutrient management system on carotenoids content:

The pooled analysis of two years data given in Table 4 and Graph 3 showed that the carotenoids content of Ashwagandha at 60, 75, 90, 105 and 120 Das (days after sowing) were also significantly affected by the integrated nutrient management system. It was observed that the values of carotenoids content increased rapidly in almost all the twelve integrated nutrient treatments with the advancement in age of plant up to 75 DAS, thereafter decreased sharply up to 120 DAS of observation. It means that 75 DAS of plant growth is the best period for the maximum synthesis of carotenoids content. The decrease of carotenoids content after 75 DAS of plant growth may be due to the decrease of nutrients supply to the plants or leaf senescence or due to the activity of carotenoids degradation enzyme of plant tissues. Amongst the integrated nutrient treatment, 50% NPK/ha + 5 tonnes FYM/ha + 3 kg Azotobactor/ha + 3 kg PSB/ha + 5 kg Zn/ha (T₁₂ treatment) resulted with significantly higher values of carotenoids content (0.49 mg/g fresh leaf weight) at 75 DAS of plant growth. This may be due to a better supply of nutrients especially nitrogen from inorganic, organic and biofertilizers sources which helped in better carotenoids synthesis led to higher values of carotenoids content of Ashwagandha. The present findings are following Ningaraju *et al.* (2018) who reported that the application of nitrogen and farmyard manure (FYM) to Ashwagandha, significantly increases carotenoids content at pre-flowering and post-flowering stages.

Table- 4 Effect of integrated nutrient management system on carotenoids b content (mg/g fresh leaf weight) at different growth stages of Ashwagandha (Pooled for two years)

Treatment	Carotenoid content (mg/g fresh leaf weight)				
	Days after sowing				
	60 DAS	75 DAS	90 DAS	105 DAS	120 DAS
T ₁	0.42	0.44	0.27	0.23	0.19
T ₂	0.42	0.44	0.27	0.24	0.19
T ₃	0.41	0.43	0.27	0.23	0.19
T ₄	0.42	0.44	0.27	0.24	0.19
T ₅	0.43	0.45	0.28	0.26	0.19
T ₆	0.45	0.47	0.29	0.26	0.20
T ₇	0.45	0.48	0.29	0.27	0.20
T ₈	0.43	0.45	0.28	0.26	0.19
T ₉	0.44	0.46	0.29	0.27	0.20
T ₁₀	0.43	0.45	0.28	0.25	0.19
T ₁₁	0.45	0.47	0.29	0.26	0.20
T ₁₂	0.46	0.49	0.30	0.28	0.21
SEm ±	0.001	0.001	0.001	0.001	0.001
CD (P=0.05)	0.004	0.004	0.002	0.002	0.002



Conclusion

Based on results obtained in the present investigation, it can be concluded that the integrated nutrient management system significantly influenced chlorophyll a, chlorophyll b and carotenoids content of Ashwagandha (*Withania somnifera* L.Dunal.) The highest values of chlorophyll a and Chlorophyll b content were recorded at 90 DAS (days after sowing) with the application of 50% NPK/ha + 5 tonnes FYM/ha + 3 kg Azotobacter / ha + 3 kg PSB/ha + 5 kg Zn/ha (T₁₂ treatment). While significant highest values of carotenoids content were recorded at 75 DAS with the application of the same treatment *i.e.*, T₁₂ treatment. 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 (T₁₂ treatment) was found to be a better-integrated nutrient management system that can be used for better quality production of Ashwagandha (*Withania somnifera* L.Dunal.) crop.

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